GROWTH HORMONE RELEASING HORMONE

A.E.M. SMALS

GROWTH HORMONE RELEASING HORMONE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF.DR. B. M. F. van IERSEL VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEDIGEN OP VRIJDAG 4 DECEMBER 1987 DES NAMIDDAGS TE 1.30 UUR PRECIES.

door

ARNOLDUS ELISABETH MARIA SMALS

geboren te Herten

Promotores Prof. Dr. A.G.H. Smals
Prof. Dr. P.W C. Kloppenborg

Co-referent · Dr. G.F.F.M. Pieters

The investigations presented in this thesis were performed in the Division of Endocrinology (Head· Prof. Dr. P.W.C. Kloppenborg), Department of Medicine (Head: Prof. Dr. A. van 't Laar), Sint-Radboud Hospital, University of Nijmegen, Nijmegen, The Netherlands.
These studies were supported by grants from the "Universitaire Onderzoekspool". Publication of this thesis has been enabled by grants from Sandoz B.V., Uden and Smals B.V., Herten.

Aan mijn
vader

CONTENTS

PREAMBLE

In 1982 the structure of growth hormone releasing hormone (GHRH) was unraveled (1,2). Subsequently it was tested in healthy normal volunteers, predominantly males (3). We wondered whether there would exist a difference in GH responsiveness to GHRH between men and women. Later we extended this study to (tall) pubertal boys and girls.

The well-known stunting of growth in children with hypercortisolism prompted us to investigate the GH response to GHRH in patients with Cushing's disease and Cushing's syndrome due to an adrenocortical adenoma.

To extend earlier studies which dealt with paradoxical reactions of GH in response to thyrotropin releasing hormone (TRH) and luteinizing hormone releasing hormone (LHRH) (4) in patients with acromegaly we performed GHRH tests in these patients, and compared the GH responses with those obtained after TRH, LHRH, somatostatin and bromocriptine.

REFERENCES

1. Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science 218 585.
2. Rivier J, Spiess J, Thorner M, Vale W (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature 300· 276.
3. Thorner MO, Rivier J, Spiess J, Borges JL, Vance ML, Bloom SR, Rogol AD, Cronin MJ, Kaiser DL, Evans WS, Webster JD, Macleod RM, Vale W (1983) Human pancreatic growth-hormone-releasing factor selectively stimulates growth hormone secretion in man. Lancet i: 25.
4. Pieters GFFM, Romeijn JE, Smals AGH, Kloppenborg PWC (1982) Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. J Clin Endocrinol Metab 54 942.

Chapter 1

REVIEW OF ANIMAL AND HUMAN STUDIES WITH GHRH

1.1 ISOLATION AND CHARACTERIZATION OF GHRH

As early as 1949 Harris (1) proposed that hypothalamic factors are secreted into the hypothalamo-hypophyseal portal circulation and regulate anterior pituitary function. In 1960 Reichlin (2) demonstrated, that lesions of the ventromedial nucleus of the rat hypothalamus resulted in impaired growth. Few years later Deuben showed that an extract of rat hypothalamus could stimulate GH release from rat pituitaries in vitro (3). In 1968 Frohman (4) reported that electrolytic lesions of the ventromedial hypothalamus in the rat decreased pituitary and plasma GH levels. In turn electrical stimulation of hypothalamic ventromedial and arcuate nuclei resulted in increased GH levels (5). In 1971 the same authors (6) demonstrated that intrapituitary administration of a partially purified extract of ovine hypothalamus in the rat led to an acute rise of GH levels. All these data pointed to the existence of a growth hormone releasing factor produced by the hypothalamus. During the next years several attempts were made to isolate and characterize this factor, none of which were, however, successful (7,8). The isolation of the growth hormone releasing hormone (GHRH) was hampered because of the minute amounts present in the hypothalamus (10-50 fmol) and the existence of rather high concentrations of a growth hormone release inhibiting factor (SRIF) in the hypothalamus. The existence of the latter factor was proposed by Kruhlich et al.(9) and later Brazeau et al.(10) isolated the hormone from ovine hypothalami while on the search for the putative GHRH and characterized somatostatin. The final solution of the problem was postponed until a rich source of a GH-releasing factor, devoid of somatostatin became available. In this respect a number of clinical observations was useful. In the sixties and seventies few authors reported cases of acromegaly caused by ectopically produced GHRH (11-15). Sönksen et al.(14) noted cure of acromegaly after removal of a bronchial carcinoid tumor. In 1980 Frohman et al.(16) partially purified and characterized a peptide with growth hormone releasing activity from extrapituitary tumors in patients with acromegaly. The limited quantity of available tissue, however, precluded further characterization. In 1982, Thorner et al.(17) from Charlottesville, Virginia, described a patient with acromegaly and Turner's syndrome, who after surgery proved to have somatotroph hyperplasia of the pituitary instead of the expected pituitary adenoma. After surgery the acromegaly remained active. An ectopic GHRF producing tumor was suspected and an islet cell tumor was found in the tail of the pancreas. After successful resection of this tumor GH levels

fell within 2 hours and subsequently the patient was cured of her acromegaly. The group of Vale (18) isolated from this so-called Charlottesville tumor a peptide with 40 aminoacids with a carboxyl terminal ($GHRH_{1-40}$). Within a few weeks Guillemin (at the same institute in La Jolla) reported the isolation of 3 peptides with growth hormone releasing properties (19,20) from a pancreatic tumor with multiple metastases obtained from a patient with acromegaly of Dr.Sassolas of Lyon (21).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly
B															
C															
D													Ile		
E															
F	His											Arg	Ile		

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A	Gln	Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg	Gln
B															
C													Asn		
D													Asn		
E													Asn		
F			Tyr						His	Glu			Asn		

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	
A	Gln	Gly	Glu	Ser	Asn	Gln	Glu	Arg	Gly	Ala	Arg	Ala	Arg	Leu	NH_2
B				Arg				Gln				Val			
C				Arg				Gln				Val			
D				Arg				Gln				Val			
E				Arg				Gln				Val			
F				Arg				Gln	Arg	Ser		Phe	Asn	OH	

FIG. 1. Amino acid sequence of A, hpGRF(1-44)NH_2; B, porcine GRF; C, bovine GRF; D, ovine GRF; E, caprine GRF; F, rat GRF

The longest peptide possessed 44 amino-acids with an amidated carboxyl-terminal. The other 2 peptides were similar in structure except for a shorter length, $GHRH_{1-40}OH$ and $GHRH_{1-37}OH$. The quantitatively major peptide was $GHRH_{1-40}OH$. Subsequently it was shown by Bohlen et al.(22) and Ling et al.(23) that in human hypothalami 2 forms of GHRH are present namely $GHRH_{1-44}NH_2$ and $GHRH_{1-40}OH$. The earlier designation human pancreatic (tumor) GHRH could now safely be changed to GHRH. Shortly after the isolation of human GHRH, hypothalamic GHRH's from other species also were identified: rat GHRH (24) which is a 43 amino acid peptide with a free carboxyl terminus and 67% homology with human $GHRH_{1-44}$, porcine GHRH (25), bovine GHRH (26), ovine GHRH (27) and caprine GHRH (27) (Fig.1). All these other peptides are composed of 44 amino acids. By molecular cloning it was demonstrated that human GHRH is processed from two precursors namely pre-pro-$GHRH_{107}$ and pre-pro-$GHRH_{108}$ (28). A putative hydrofobic signal peptide of possibly 20 amino acids is joined to a pro-GHRH, 87 or 88 aminoacids long (28). The gene encoding human GHRH is located on chromosome 20 (29).

1.2 DISTRIBUTION OF GHRH

1.2.1 GHRH in normal tissues

Neuronal cell bodies producing GHRH have been characterized by immunohistochemistry, using antibodies against $GHRH_{1-40}$ and $GHRH_{1-44}$ in the arcuate and the ventromedial nuclei (30-32) of human hypothalami. GHRH staining cell fibers are mainly found in the external layer of the median eminence. These fibers are in close contact to the capillary loops that coalescence to form the portal vessels. In human hypothalami highest concentrations of immunoreactive (IR) GHRH were found in the arcuate nucleus (83 ± 4 ng/ml protein). Lower quantities were present in other hypothalamic regions. In the upper portion of the pituitary stalk the highest concentrations of IR GHRH were found (1454 ± 48 ng/mg protein), whereas rather low levels were found at the distal end (121 ± 3 ng/mg). This concentration gradient suggests, that the peptide reaches the anterior pituitary mainly via the long portal vessels. Somatostatin has a pattern of distribution along the pituitary stalk very similar to GHRH (33). Also in other species the presence of GHRH is restricted to only few areas in the brain, i.e. in rats in the arcuate, paraventricular and dorsomedial nuclei and around the ventromedial nucleus (34-39), in the monkey in the arcuate and ventromedial nucleus (40-43) and in the cat in the mediobasal hypothalamus (44). Phylogenetically of interest was the demonstration by immunohistochemistry of GHRH in the nervous system of Aeshua Cyanea, an insect (45). Bosman et al.(46) found GHRH-like immunoreactivity (measured by a specific RIA for $GHRH_{1-44}$) in appreciable amounts in extracts of the normal human

pancreas. Lower concentrations were found in extracts of the thyroid, lung, stomach, duodenum, ileum, colon, adrenal, kidney and the placenta (47-49). It has to be added, however, that definite characterization of this GHRH awaits further evidence. In this context it is of interest to note that as far as pancreatic secretion of GHRH is concerned Kashio et al.(50) reported IR-GHRH in blood shortly after glucose loading in healthy volunteers.

1.2.2 GHRH in tumorous tissues

Ectopic production of GHRH causing acromegaly has been described above. Besides the earlier mentioned pancreatic tumors that produce GHRH, Roth et al.(51) very recently reported a patient with coexisting acromegaly and pheochromocytoma, which produced $GHRH_{1-44}$. GHRH-like immunoreactivity was also demonstrated in some small cell carcinomas of the lung, medullary carcinomas of the thyroid, gastrinomas, glucagonomas, insulinomas and thymic and bronchial carcinoid tumors (47,52,53). Excessive production of GHRH with clinical acromegaly has been occasionally described in hypothalamic gangliocytomas or hamartomas (54).

1.2.3 GHRH and colocalization with other neuroactive substances

Colocalization of GHRH and dopamine (55) and of GHRH with tyrosine hydroxylase-like immunoreactivity (56,57) has been demonstrated in rat arcuate nucleus. These data point to a possible interaction between GHRH and dopamine in the hypothalamus. Coexpression of GHRH-like immunoreactivity and α-melanotropin-like immunoreactivity is present in some interneurones of rat lateral dorsal hypothalamus (58). Fuxe et al.(59) put forward the medianosome hypothesis-concept. The medianosome is defined as an integrative unit, which consists of well-defined aggregates of transmitter identified nerve terminals, interacting with one another in the external layer of the median eminence. These authors found the existence of putative medianosomes in rat hypothalami, consisting predominantly of GHRH nerve terminals (costoring dopamine) as well as of SRIF and dopamine nerve terminals. Other authors reported that GHRH-positive cell bodies in the arcuate nucleus also contained neurotensin and/or galanin-like immunoreactivities (38).

1.3 IN VITRO STUDIES WITH GHRH

1.3.1 In vitro studies with pituitary tissue

Binding of GHRH to specific receptors has been reported for bovine anteri-

or pituitary membranes (60), rat pituitary membranes (61) and intact rat pituitary cells in culture (62). Glucocorticoids enhanced GHRH receptor capacity (62). The number of GHRH binding sites was dramatically decreased after adrenalectomy and restored after chronic dexamethasone treatment (61). Recently Ceda et al.(63) reported that short term (< 4 h) incubation of cultured rat pituitary cells with dexamethasone inhibited GHRH-induced GH secretion, whereas longer incubations with the glucocorticoid enhanced both basal and GHRH-stimulated GH release. It is likely that thyroid-hormones can modulate GHRH receptors on somatotrophs (64). To our knowledge, however, direct measurement of GHRH binding sites in hypothyroidism has not been reported.

In primary cultures of dispersed rat anterior pituitary cells, GHRH specifically stimulated release of GH (18,19,65-72). No effect of physiological doses of GHRH upon the secretion of β-endorphin, follicle-stimulating hormone, luteinizing hormone, thyrotropin or prolactin has been reported. The dose of GHRH to elicit half maximal release of GH (ED50) ranges from 0.5 to 1x 10^{-11}M. This is a concentration comparable to the basal hypophyseal portal blood levels of other known hypothalamic hypophysiotropic hormones (73-75). At a concentration of 10^{-6}M GHRH, prolactin release in rat pituitary halves was slightly stimulated (76). In perifused rat pituitary cells, however, $GHRH_{1-44}$ stimulates release of stored prolactin without altering the release of newly synthesized prolactin (77). In perifused rat anterior pituitary cells it has also been shown that the action of GHRH on (stored) GH release is very rapid i.e. within 1 minute (65,70, 77-79). Stimulation of newly synthesized GH was rather shallow, requiring prolonged exposure to GHRH (77). The in vitro effects of GHRH on GH release by human pituitary tumor tissues have also been investigated (80-83). In GH secreting adenomatous pituitary tissues, GHRH is capable of releasing GH. No studies have been reported on the effect of GHRH on normal human somatotrophs.

1.3.2 Mechanism of action of GHRH on somatotrophs

Many hormones produce their biological effects by interfering with receptors on the intramembranous adenylate cyclase system. A stimulatory effect of GHRH on adenylate cyclase has been reported (84-86). GHRH increases both extra- and intracellular cAMP accumulation (66,67,70,84-88). Extracellular calcium is required for GHRH-stimulated GH release (66,68). Incubation of normal rat pituitary cells with the calcium entry blocker verapamil attenuates the GH response to GHRH (68). Cobalt (66,68) and inhibitors of calcium-binding proteins (85) have a similar effect. Login et al. demonstrated rapid stimulation of calcium influx by GHRH, concomitantly with stimulation of GH release in rat anterior pituitary cells (89). There

is also a role for calmodulin, the Ca-receptor binding protein, in the GHRH-induced activation of adenylate cyclase. It has been demonstrated that a calmodulin antagonist abolishes GHRH-induced GH secretion and cAMP accumulation (85,90). In contrast, other authors found that a calmodulin antagonist increased the GHRH induced GH and cAMP levels (91)

A role of prostaglandins in GH secretion has been indicated by demonstrating that GHRH-induced GH release in vitro can be inhibited by the cyclooxygenase inhibitors aspirin and indomethacin (92). Other authors found no suchlike inhibition with indomethacin (93). GHRH stimulates GH and arachidonic acid release from rat anterior pituitary cells (94). Thus, while an interaction exists between prostaglandines, GHRH and GH, the precise relationships remain to be classified.

The relative contributions of the calcium-calmodulin system, the eicosanoid cascade and the phosphatidylinositol-protein kinase-C pathway (69, 95-97) in the regulation of GH secretion by GHRH remains to be determined.

Prolonged perifusion of somatotrophs with GHRH not only results in immediate release of stored GH but also in synthesis of new GH (77). GHRH stimulates transcription of the GH gene and increases GH messenger RNA in rat pituitary cells (98).

1.3 3 Interactions of GHRH in vitro with other substances

GH secretion is regulated principally by two factors, GHRH stimulates and somatostatin (SRIF) inhibits the release and/or synthesis of GH by the somatotroph. Unlike the other trophic hormones GH has no endocrine target organ. The peripheral effects of GH are mediated by somatomedins or also called insulin-like growth factors (99-101). In man at least 2 major forms of somatomedins occur: insulin-like growth factor I or somatomedin-C (Sm-C) and insulin-like growth factor II or somatomedin A (Sm-A). Highly purified Sm-C can inbibit GHRH-stimulated GH release of rat pituitary cells in vitro (102). Recently it was demonstrated that Sm-A is a less potent inhibitor of GHRH-induced GH release than Sm-C (103). In rat anterior pituitary cells in vitro, SRIF (SRIF 14 and SRIF 28) inhibits the GH response to GHRH in a non-competitive way (65,68,72,104,105). Thus GHRH and SRIF act via different receptors on the somatotrophs. GHRH has been shown to stimulate SRIF release from median eminence fragments in vitro (106). Subsequently it was shown that GHRH does so via β-endorphin (107). Kraicer et al.(108) demonstrated that the timing of the episodic bursts of GH secretion is set by SRIF withdrawal, whereas the magnitude of the bursts is determined by the amount of GHRH impinging on the somatotrophs before and during SRIF withdrawal in perifused rat pituitary cells. There is also evidence that GH and Sm-C stimulate SRIF release from rat hypothalamus in vitro (109,110). The possibility remains that somatomedins

directly inhibit hypothalamic GHRH release. As far as we know no studies addressing this question have been reported in literature. It is well-known that chronic GH excess by implantation of a GH-producing tumor in rats reduces hypothalamic GHRH content (111). This effect might be mediated by GH itself or by increased somatomedin production induced by GH. All these data point to very complex feedback mechanisms between GHRH, SRIF, GH and the somatomedins. This is also illustrated by data of Andhya et al.(112) and Katakami et al.(113).

Thyroid hormones play an important role in the regulation of GH secretion. Recently it has been reported that cultured anterior pituitary cells from hypothyroid rats have a reduced maximal GH response to GHRH (64). Triiodothyronine pretreatment enhances the response to GHRH of euthyroid rat pituitary cells in vitro (72). Dexamethasone pretreatment in the same way augments the GH response to GHRH at least in vitro (63,72, 114,115). Borges et al.(78) observed that prior exposure of rat pituitary cells to GHRH at maximal concentrations enabled TRH to become a secretagogue of GH at the pituitary level, whereas TRH did not release GH by itself. The authors suggest that this might be the mechanism underlying the paradoxical GH response to TRH observed in some acromegalics. To our knowledge this study has not been performed with GH-secreting adenomatous pituitary tissue from patients with acromegaly.

1.3.4 Extrapituitary effects of GHRH

A preliminary report showed that GHRH stimulates release of neurotensin and cAMP from a line of rat C-cells (116). In a detailed study it was demonstrated that GHRH also stimulated calcitonin release from the same cell line (117).

Laburthe et al. reported that GHRH acts like vasoactive intestinal peptide (VIP) on human intestinal epithelial membranes (118). However, the very high concentrations of GHRH necessary to produce such effect make a physiological role of GHRH on intestinal membranes less likely. Recently rat GHRH has been shown to stimulate amylase release in a preparation of dispersed acini of a guinea-pig pancreas (119), probably by interacting with VIP receptors. In the dog, extrapituitary effects of GHRH on the endocrine pancreas have been demonstrated (120,121). These authors demonstrated that GHRH produces a dose-dependent increase in insulin, glucagon and somatostatin from the isolated dog pancreas. In the human extrapituitary effects of GHRH so far have not been reported.

1.4 EFFECTS OF GHRH IN VIVO IN ANIMALS

1 4.1 Effect of iv GHRH on GH secretion in rats

The first studies of GHRH in vivo were done in rats anaesthetized with sodium pentobarbital, which is known to inhibit the release of endogenous GHRH and SRIF (122). Human $GHRH_{44,40}$ and $_{-37}$ proved to be equally effective in releasing GH (123) The GH secretion induced by $GHRH_{1-44}$ was dose-dependent in the range from 50 ng to 1 µg. Maximum GH concentrations were achieved within 3 to 5 minutes following iv administration of GHRH. Baseline levels were reached 30 minutes following treatment. Later the same authors reported that maximum release of GH in the pentobarbital-anaesthetized rats was obtained with 5 µg/kg GHRH (124,125). In the same study no change in the pituitary GH response to GHRH was found with increasing age of the rats (124) which is in contrast to data of Ceda et al.(126), showing a diminished pituitary responsiveness to GHRH in aging male rats in vivo as well as in vitro. Sonntag et al.(127), however, reported an age related impairment of the GH response to GHRH occurring in vivo, but not in vitro. There is no explanation for these discrepant results.

When GHRH was given to conscious, freely moving rats, GH answers were highly variable, increases of GH only being observed in 30% of animals tested (128). When the animals, however, were pretreated with antibodies raised against somatostatin, the heterogeneity in GH responses could be completely eliminated (128). In the same study the animals were subjected to a 72-hour fast, a situation characterized by increased SRIF production (129). Administration of GHRH to these animals did not result in increased GH levels. Responsiveness could be restored by pretreating the animals with anti-SRIF (128). The same authors (128) also demonstrated that in freely moving rats the well-known pulsatile secretion of GH (130) could be completely deleted by treating the animals with antibodies raised against GHRH. When the endogenous rat GHRH and SRIF were eliminated by appropriate antibodies, human GHRH injected iv every hour was able to produce virtually reproducible GH responses (131,132). The dose-response curve of GHRH proved to be the same as previously reported in anaesthetized rats.

All these data point to reciprocal effects of GHRH and SRIF in the regulation of GH secretion. The interaction between GHRH and SRIF in vivo was nicely demonstrated by Plotski and Vale (133) measuring immunoreactive GHRH and SRIF levels in the hypophyseal portal blood of rats. They found that each GH secretory episode is initiated by pulsatile secretion of GHRH into the portal circulation which is preceded by or concurs with a moderate reduction of inhibitory tone provided by portal SRIF. This study demonstrates that SRIF and GHRH are secreted in an oscillatory fashion and that both are 180 degrees out of phase with respect to each other.

The interaction between GHRH and SRIF is further delineated by the fact that intracerebroventricular (icv) injection of a small dose of GHRH in conscious male rats causes a paradoxical decrease in GH secretion as seen by a suppression of the subsequent GH pulse (134). Prior iv injection of anti-SRIF completely blocked the GH-suppressive effects of icv GHRH indicating that icv GHRH probably acts by increasing SRIF secretion. In an elegant study Miki and Ono (135) very recently showed that the decline in hypothalamic SRIF content, induced by treating rats with cysteamine, a potent depletor of SRIF, was accompanied with increased GHRH release. The authors speculated that SRIF withdrawal may be involved in triggering the episodic GHRH release in normal physiology.

1.4.2 Sex difference in GH responsiveness to GHRH in rats

There is a striking difference in GH secretion between male and female rats (130). Male rats have GH pulses occurring every 3 to 3.5 hour with peak GH levels of several hundred nanograms per ml. Between the pulses GH levels are low or undetectable. Female rats, however, exhibit a more stable GH secretion pattern with lower GH peak values and higher GH values between the peaks. The GH response to GHRH in vivo is reported to be significantly higher in adult male rats than in adult female rats (125). This difference became clear after puberty (125). Gonadectomized male rats had a similar GH response to GHRH as adult female rats. When the intact and gonadectomized rats were pretreated with testosterone a significant enhancement of GH responsiveness to GHRH was seen. However, treating gonadectomized female rats with 17-β-estradiol did not change the GH response to GHRH (136). In vitro, no modulating effects of androgens or estrogens on GH response to GHRH was observed (136). This is in contrast to data of Evans et al.(137) who showed that exposure of rat anterior pituitary cells to testosterone or estrogen enhances respectively diminishes the GH response to GHRH. In in vitro perifusion studies of anterior pituitaries from gonadectomized male rats treated with testosterone Ohlsson et al. (138) showed the same enhancing effect of androgens on GHRH-induced GH release.

Part of the sex difference in GH secretion can be explained by the greater number of somatotrophs in the male rat as compared to female rats (139) and the greater GH secretory capacity and sensitivity to GHRH in the former (139-141). Furthermore the sexual dimorphism in GH secretion is determined by the complex interactions between GHRH and SRIF. The data could be put together by hypothesizing that in male rats, GHRH and SRIF are episodically released at 3 to 3.5 h intervals, with maximal GHRH secretion during GH peaks, while SRIF release is highest during GH troughs. In female rats, GHRH and SRIF secretion are more constant (97,142). Shulman

et al.(143) reported that high doses of estrogens (5 and 50 μg) inhibited the GH secretory response to GHRH in castrated adult female rats in vivo and decreased Sm-C levels, whereas low doses (0.05 and 0.5 μg) increased Sm-C concentrations without altering GH responsiveness to GHRH. In vitro, Fukata and Martin (144) could not demonstrate an influence of testosterone, dihydrotestosterone and 17β-estradiol on GHRH-induced GH release in rat anterior pituitary cells.

1.4.3 Continuous versus intermittent exposure to GHRH

Wehrenberg et al.(145) demonstrated that continuous infusion of a large dose of GHRH to unanaesthetized rats increased plasma GH levels for several hours. This increase was followed by a progressive decline in GH despite continuation of the infusion. The counterregulatory effects of SRIF were eliminated by pretreating the rats with an antiserum raised against SRIF. After 24 hours of GHRH infusion the rats received a bolus injection of GHRH. At this time, when 80% of the pituitary GH stores were depleted, the acute bolus injection of GHRH proved to be less effective in releasing GH than in control rats. In another study male rats, not pretreated with anti-SRIF, were exposed to a continuous infusion of a high dose GHRH for 8 or 31 hour (146). It appeared that the pulsatile secretion of GH in these animals was preserved. With this treatment the frequency of GH pulses was not altered, peak GH levels, however, were increased. The author explained the seemingly conflicting data by claiming that hypothalamic SRIF must be responsible for the intermittent secretion of GH during a continuous infusion of GHRH. Furthermore this author suggests that some desensitization or down-regulation of GHRH receptors or depletion of a readily releasable GH pool must have occurred, as the mean GH levels during the 8-hour infusion were higher than during the 31-hour infusion of GHRH. The same explanation - desensitization and/or depletion of readily releasable GH pools - can be given for the observed decrease in GH responsiveness to a bolus injection of GHRH after continuous infusion of GHRH (145).

Intermittent administration of GHRH to rats enhances the GH response to GHRH (147). It has also been reported that pulsatile but not continuous infusion of GHRH enhances the pituitary GH content and growth in male rats with induced GHRH deficiency and in normal female rats (148). There is no ready explanation for the observed differences in GH secretion between intermittent and continuous administration of GHRH. It could be attributed to differences in the GH synthesis/release ratio or to different levels of GHRH-receptor down-regulation by the respective modes of administration of GHRH. Thus, in vivo, no final proof for the occurrence of GHRH desensitization has been given.

In this context it seems appropriate to discuss also the in vitro data

of the surmised GHRH down-regulation. Chronic exposure of cultured rat anterior pituitary cells to GHRH causes partial loss of GH responsiveness to GHRH (149). Seiffert et al.(150) demonstrated that pretreatment of rat anterior pituitary cells with GHRH leads to a down-regulation of the GHRH receptor, as the binding capacity of the GHRH receptor for GHRH was reduced with 40 to 70% without affecting the affinity for GHRH. Other authors, however, found no evidence of desensitization (151). Evidence in favour of down-regulation of GHRH in vitro was given by Simard and Labrie (152) and by Ceda and Hoffman (153). The precise mechanisms underlying the in vivo and in vitro observed desensitization, however, need to be clarified. Very recently it was shown that SRIF co-incubation partially prevented the down-regulation of GHRH receptors of rat anterior pituitary cells in culture pretreated with GHRH (154).

1.4.4 Interaction of GHRH with biological substances and pharmacological agents

Before the structure of GHRH was unraveled, Eden et al.(155) reported that clonidine, an α-agonist, stimulates GH release in adult male rats probably by enhancing the GHRH secretion, not by inhibition of the somatostatin release, as clonidine was still able to release GH when the animals were pretreated with anti-SRIF. These results were later confirmed by other authors (156-158).

In adult male rats pretreatment with the cholinergic antagonist pirenzepine and atropine significantly reduced the rise in GH induced by GHRH whereas pretreatment with the cholinergic agonist pilocarpine potentiated it (159). In vitro no effect of atropine or pilocarpine on GHRH-induced GH secretion was observed (159). Kakucska and Makara (160) found indirect evidence for a stimulatory role of acetylcholine on GHRH release by infusion of acetylcholine into the third ventricle of rats, in which somatostatinergic innervation of the median eminence was surgically cut off. In control rats, however, acetylcholine had no such an effect.

There is also evidence that γ-aminobutyric acid (GABA) has a role in secretion of GH, as icv injection of GABA increases plasma GH in conscious freely moving rats (161). Pretreatment of the rats with an antiserum raised against GHRH abolished GH release by GABA, indicating that GABA acts via GHRH. Fiók et al.(162), however, adduced evidence for an inhibitory influence of GABA activity on the secretion of GHRH.

Opioid peptides induce GH secretion by stimulating GHRH release (163). A similar conclusion was reached by Wehrenberg et al.(164).

Also the GH secretion induced by serotoninergic mechanisms in the rat is - at least in part - mediated by GHRH (165).

Hyperglycemia has been reported not to influence the GH release induced

by GHRH in anaesthetized rats (166). Locatelli et al.(167) described the effect of GHRH on GH secretion in male rats with diabetes induced by streptozotocin. They found increased pituitary GH responsiveness to GHRH in this model of diabetes. No ready explanation was given by the authors for this remarkable finding (cf. paragraph 1.6.4.). Infusion of free fatty acids significantly blunted the GH release after GHRH, probably by increasing SRIF secretion (166).

The influence of thyroid hormones on GH secretion has been studied in thyroidectomized rats. GH secretion, pituitary content of GH and GH responsiveness to GHRH are significantly diminished in thyroidectomized rats (168). Hypothalamic GHRH content was also reduced. Cultured pituitary cells of thyroidectomized rats exhibited reduced GHRH sensitivity while the suppressive effects of SRIF on GH secretion were increased (168). Thyroxine replacement completely restored hypothalamic GHRH content and spontaneous GH secretion, whereas pituitary GH content and sensitivity to GHRH and SRIF in vitro were only partially restored. A similar conclusion was reached by Root et al.(169). These authors also studied the effect of short term administration of large amounts of thyroxine to rats on GH responsiveness to GHRH in vivo. They found no difference in GH response as compared to controls (169).

Very recently Tannenbaum (170) reported that glucocorticoids potentiate GH responsiveness to GHRH in vivo in rats during peak but not during trough periods of the GH rhythm. This contrasts with studies in patients with Cushing's disease, in whom the GH response to GHRH is blunted or even deleted (Chapter 3).

From data mentioned above it ensues that GHRH secretion is influenced by α-adrenergic, cholinergic, serotoninergic, GABA-ergic and opioid factors and by glucocorticoids. Furthermore thyroid hormones are necessary for full activity of GHRH. Recently it was also demonstrated that GHRH produced a significant decrease in melatonin levels in pentobarbital anaesthetized adult male rats (171). On the other hand melatonin pretreatment blunted the GH response to GHRH. Thus an inverse relationship exists between GH and melatonin. A similar reciprocal relation has been reported for GH and calcitonin (CT) (172). These authors demonstrated that the suppression of GH levels found after icv injection of CT in rats could not be restored by pretreating the animals with anti-SRIF. GHRH injection one hour after icv CT failed to stimulate GH levels.

1.4.5 Other biological effects of GHRH

Vaccarino et al.(173) icv administered rat GHRH or human GHRH to fasted c.q. hungry rats and observed an increase in food intake as compared to vehicle injection. Peripheral injection of GHRH had no effect on food

intake, suggesting a centrally mediated effect of GHRH on eating behaviour. Imaki et al.(174), however, reported the opposite after icv injection of GHRH to rats, i.e. a suppression of starvation induced feeding. Ehlers et al.(175) studied the effects of icv GHRH on electroencephalographic (EEG) and behavioral signs of sleep and wakefulness in adult male rats. An increase in slow wave sleep and corresponding EEG changes was reported after icv GHRH.

Clark and Robinson (148) demonstrated increased pituitary GH content and corresponding accelerated growth in female rats by long-term pulsatile infusions of the active core of human GHRH ($GHRH_{(1-29)}NH_2$). Continuous infusion of GHRH, however, had no effect on growth and pituitary GH content. An indirect way of assessing the role of GHRH in the regulation of growth was reported by Hammer et al.(176). These authors could produce a strain of mice, containing in their genome the coding region of the human GHRH gene. Expression of this gene in the animals resulted in measurable levels of GHRH and increased the concentrations of mouse growth hormone, which in turn accelerated growth rates relative to those in control mice.

Accary et al.(177) described an extrapituitary effect of GHRH by demonstrating an increased gastrin secretion after subcutaneous administration of the peptide. Hermansen et al.(120,121) reported dose dependent GHRH-mediated increases of insulin, glucagon and somatostatin from the isolated dog pancreas. A large dose of GHRH was able to stimulate exocrine pancreatic secretion of the rat in vivo (178). Twice daily subcutaneous injections of rat GHRH to 24-day-old rats for 14 days increased gastric fundus weight concomitantly with DNA, RNA and protein contents, producing hyperplasia and hypertrophy within this gland (179).

1.5 STUDIES WITH GHRH IN HEALTHY SUBJECTS

1.5.1 Studies in normal men and women

The first clinical study with GHRH in healthy volunteers was reported by Thorner et al.(180). Six normal young men received an iv bolus injection of $GHRH_{1-40}$ (1 µg/kg) or placebo. All subjects had an increase in serum GH levels, whereas the concentrations of the other anterior pituitary hormones and the gastro-intestinal hormones did not change. There was a great variability in the GH responses. Dose-response relationships of GHRH were studied by Vance et al.(181). GHRH doses of 0.1, 0.33, 1.0, 3.3 and 10 µg/kg body weight were given to 12 normal men. No relationship was found between the dose and the maximal GH answer. The two higher doses of GHRH, however, resulted in a more prolonged and in a biphasic pattern of GH release. Peak GH levels were reached within 30-60 minutes after GHRH. Again there was a great inter-subject variability. The only side effect

was a transient facial flushing. A dose of 1 μg/kg $GHRH_{1-44}$ was reported by Gelato et al. to be maximally effective in releasing GH in normal young adult men and women (182). A similar conclusion was reached by Losa et al. reporting 50 μg $GHRH_{1-44}$ or 1 μg/kg to be most effective (183). Sassolas et al.(184) concluded that 80 μg $GHRH_{1-44}$ is the optimal dose for GH release. In contrast to the data in rats, Gelato et al.(182) did not find a statistically significant difference in GH responsiveness to GHRH between men and women. We, however, found significantly higher GH responses to GHRH in young adult men than in young adult women (Chapter 2). In Chapters 2.1 and 2.2 and in the final section of this thesis we further comment these findings. Two studies have been performed addressing the question whether GH responsiveness to GHRH changes during the menstrual cycle Evans et al.(185) denied such influence, Egli et al.(186), however, reported that the GH response to GHRH was the lowest during the luteal phase of the menstrual cycle.

1.5.2 Kinetic aspects of GHRH in normal subjects

Frohman et al. calculated the metabolic clearance rate (MCR) and the plasma disappearance rate ($t_{\frac{1}{2}}$) of $GHRH_{1-40}$ after a bolus injection and continuous infusion with different doses of the peptide (187) GHRH levels were measured by radio-immunoassay. The MCR was 194 ± 17.5 liter/m^2 per day. The disappearance rate after the bolus injection could be divided in two linear phases: an equilibration phase (7.6 ± 1.2 min) and a subsequent elimination phase (51.8 ± 5.4 min). The latter value was not different from that obtained after terminating the continuous infusion. A similar conclusion was reached by Sassolas et al. for $GHRH_{1-44}$, reporting a $t_{\frac{1}{2}}$ of the distribution phase of 6.8 ± 0.4 min and of the elimination phase of 93 ± 2.98 min (184). The reported $t_{\frac{1}{2}}$ of GHRH is much longer than for other hypothalamic hormones (TRH, LHRH, SRIF) which have $t_{\frac{1}{2}}$ values ranging from 1 to 10 min (188-190). Very recently Frohman et al.(191) by means of high performance liquid chromatography (HPLC) demonstrated that $GHRH_{1-44}$ is very rapidly enzymatically degradated to a biologically inactive cleaving product ($GHRH(_{3-44})NH_2$), which still is measured in the radio-immunoassay and could account for the spuriously long $t_{\frac{1}{2}}$ of the elimination phase earlier mentioned. This author concluded that the real $t_{\frac{1}{2}}$ of $GHRH_{1-44}$ is 6.8 min, well in line with the $t_{\frac{1}{2}}$ of other hypothalamic hormones. Until now, no such study has been published for $GHRH_{1-40}$.

1.5.3 Influence of age on GH responsiveness to GHRH

There is controversy in literature regarding the influence of age on the GH response to GHRH in men. Shibasaki et al.(192) reported a lower or even

complete absence of the GH response after $GHRH_{1-44}$ in males over forty years old Pavlow et al.(193), however, did not find age-dependent alterations in the magnitude of GH responses to GHRH in healthy aging American men. It is not known whether dietary, cultural or other differences between American and Japanese men may account for the observed discrepancies As far as the prepubertal and pubertal age is concerned Schriock et al (194) and van Vliet et al (195) found that GH responsiveness to GHRH did not differ from that in adults. The interpretation of the preliminary results at young age is seriously hampered by the low numbers of youngsters in the successive studies We further comment on this aspect of GH responsiveness to GHRH in Chapter 2.2 and in the final comments

1.5 4 Hypothalamic hormones, neurotransmitters, drugs and other agents which interact with GH responsiveness to GHRH

Under conditions of hyperglycemia GH responsiveness to GHRH in normal subjects is significantly diminished in comparison with the euglycemic state (203-205). Raised levels of free fatty acids (FFA) have a similar inhibitory effect on GH responsiveness to GHRH (206). These effects of FFA and glucose are probably mediated by SRIF. Therefore, it has to be emphasized that studies on interaction of GH responsiveness to GHRH by other agents, neurotransmitters, other releasing hormones, drugs, etc. have to be performed in the fasting state.

Sartorio et al.(207) administered to 10 normal men placebo, TRH, GHRH and simultaneously GHRH plus TRH on four separate occasions. TRH did neither stimulate GH release nor did it augment the GH response to the combined test. When, however, GHRH was given as 3 consecutive boli at 2-hour intervals (at a dose of 25 μg) followed by a fourth injection of either GHRH (25 μg), TRH (200 μg) or placebo it appeared that TRH caused a small but significant increase of the GH response as compared to the placebo. The authors suggest that GHRH exposure might contribute to the GH-releasing activity of TRH in some pathological conditions, showing a paradoxical GH response to TRH.

In a number of studies combinations of hypothalamic releasing hormones (TRH, CRF, LHRH and GHRH) have been administered as bolus injections. Sheldon et al.(208) found no apparent inhibition or synergism of the releasing hormones when they were given as a bolus compared to the responses after testing each releasing hormone separately. In contrast Looy et al (209), with a similar study protocol found potentiation of the TSH response. These latter results were confirmed by Cohen et al.(210). Combined administration of GHRH and insulin elicited a significantly higher GH response than either agent alone, supporting the hypothesis that GHRH and insulin-induced hypoglycemia release GH via different pathways

which are at least in part additive.

There is ample evidence that the cholinergic system is involved in GH secretion as blockade of GHRH-induced GH release is seen after pretreatment of normal men with cholinergic antagonists such as atropine or pirenzipine (211-214).

Dopaminergic and α-adrenergic blockade had no influence on the GH response to GHRH (213). Chihara et al.(200), however, found that L-Dopa stimulates GHRH release in humans. These data were confirmed and extended by Vance et al.(215) suggesting that dopamine infusion inhibits SRIF secretion, which allows GHRH to have a greater stimulatory effect on GH secretion. In contrast, Giusti et al.(216) reported that the simultaneous administration of GHRH and domperidone, an anti-dopaminergic drug, resulted in a more marked GH increase than GHRH alone, suggesting that the dopaminergic tone may play an inhibitory role on GH secretion in man. The β-adrenergic blocker propranolol enhances GH-secretion in response to GHRH in prepubertal children (217). Probably this effect is mediated by inhibition of SRIF-release.

In addition to the complex network of neurotransmitters and neuropeptides involved in the control of GH secretion, the pineal gland plays a major role in the regulation of GH in rodents (218). In humans oral melatonin administration has been demonstrated to enhance the GH response to GHRH probably at the hypothalamic level by modulating GHRH or SRIF (218).

1.5.5 Feedback mechanisms

Rosenthal et al.(219) studied the effect of pretreating six normal men with twice daily GH injections for five days on GH secretion induced by GHRH. After such treatment, a blunted response to GHRH was seen. In theory the reason for this blunting might be i) increased somatomedin levels, ii) increased somatostatin secretion or iii) a direct effect of GH on the somatotrophs. Very recently Ross et al.(220) demonstrated that an iv injection of GH 3 hours prior to the GHRH test completely abolished the GH response to GHRH. This effect could not be mediated by somatomedin-C, as this growth factor was still not raised by the prior GH administration. Ross et al.(220) could not precisely delineate whether increased SRIF secretion or a direct effect of GH on the somatotrophs must be held responsible for the attenuated GH response to GHRH. These authors adduced some evidence that increased SRIF secretion as a cause was less likely as they did not observe such attenuation in children with extensive hypothalamic lesions and panhypopituitarism in whom they considered such increased SRIF secretion unlikely. Thus GH may regulate its own secretion independent of changes in Sm-C and SRIF. Very recently Hanew et al.(221) reported similar blunting of the GH response to GHRH in GH-deficient children with presumed

hypothalamic GHRH-deficiency given biosynthetic GH for 2 days. The authors therefrom deduced that GH blunts the GH response to GHRH through a direct effect on the somatotroph.

1.5.6 Continuous versus pulsatile administration of GHRH to normal subjects

Gelato et al.(222) administered a continuous infusion of $GHRH_{1-44}$ (1 µg/kg/hr for 4 hours) to 15 young adult men. Peak GH levels were reached within 60-90 min and then fell progressively, but did not return to baseline levels. A GHRH bolus injection at the end of the continuous infusion showed a markedly attenuated GH response compared to the response after saline infusion. Like in rats this effect could be mediated by receptor desensitization or down-regulation, depletion of a readily releasable GH pool, a combination of both or by the counter regulatory rise in SRIF secretion. Vance et al.(223) demonstrated that continuous GHRH infusion during 6 and 24 hour resulted in augmentation of pulsatile GH secretion. A supramaximal bolus injection of GHRH at the end of the infusion, however, resulted in an attenuated GH response as compared to placebo. The data were interpreted to mean that there is a limited readily releasable GH pool and/or partial refractoriness of the somatotroph after prolonged exposure to GHRH. To further resolve this issue the same authors administered to normal subjects GHRH in a continuous infusion during 6 hours, and 5,5 hour after starting the infusion either a supramaximal GHRH bolus injection or insulin was given (224). The greater GH secretion was seen with the combination GHRH infusion plus insulin-induced hypoglycemia. The GHRH bolus injection after continuous infusion of GHRH resulted in a diminished GH response. The data suggest that the somatotrophs become partially refractory to GHRH after prolonged exposure to GHRH, probably by partial desensitization or down-regulation. Evidently, pituitary GH stores are not depleted as the somatotrophs are still responsive to insulin-induced hypoglycemia. A similar conclusion was reached by other authors (225). Decreased GH responsiveness to GHRH also has been reported to occur in normal subjects after repetitive or pulsatile administration of the releasing hormone (226,227). Restoration of the GH response could be accomplished by pretreating the subjects with pyridostigmine an inhibitor of cholinesterase and thus a cholinergic agonist (227). Therefrom the authors deduced that the decrease in GH responsiveness after pulsatile injection of GHRH may be due to inhibition of the SRIF release induced by the first GHRH pulse.

So far, the limited experience with continuous and pulsatile administration of GHRH in healthy subjects does not allow to draw firm conclusions on the mechanism of decreased GH responsiveness to GHRH. Each of the

three mechanisms delineated in paragraph 1 5.5. must be kept in mind when interpreting GHRH studies either of the continuous or intermittent type. It does seem highly improbable that depletion of pituitary GH stores might be held responsible for the described phenomena.

1.5.7 Plasma GHRH levels in normal subjects

Penny et al.(196) developed an assay for measurement of GHRH-immunoreactivity in peripheral plasma. The antiserum used equally binds $GHRH_{1-44}$, $GHRH_{1-40}$, and $GHRH_{1-37}$. Normal levels were reported to vary from < 10 to 60 ng/l. These authors subsequently reported that immunoreactive GHRH levels in normal subjects increased after ingestion of a mixed breakfast, suggesting a peripheral source of GHRH (50,197). Very recently they demonstrated an increase in circulating GHRH levels after oral fat or protein, but not after oral carbohydrate, insulin-induced hypoglycemia or iv arginine (198). No correlation was found between spontaneous pulses of GH secretion and plasma GHRH levels, further suggesting the release of GHRH from a source outside the hypothalamus Knip et al.(199), however, demonstrated that in normal children GHRH peaks preceded or coincided with the increase in GH secretion in 65% of the sleep related GH increments.

Chihara et al.(200) measured immunoreactive GHRH levels using an antiserum specifically directed against $GHRH_{1-44}$. Plasma GHRH levels ranged from 2.8 - 18 1 ng/l and increased after administration of oral L-Dopa. In patients with hypothalamic lesions circulating GHRH levels were similar to those of normal subjects, indicating that the source of plasma GHRH is not solely the hypothalamus (50,201). Andhya et al.(112) reported plasma $GHRH_{44}$ levels in normal males, 21 - 39 yrs, ranging from 6.3 to 14 ng/l. Penny et al.(202) studied the distribution of the different molecular forms of GHRH in plasma by using HPLC. $GHRH_{1-40}$ was shown to be the predominant circulating molecular form, while $GHRH_{1-44}$ showed the greatest increase after a mixed meal. Furthermore, $GHRH_{1-37}$ and a yet unidentified factor, probably $GHRH_{1-42}$ were found.

1.6 CLINICAL STUDIES WITH GHRH IN PATHOLOGICAL CONDITIONS EXCEPT ACROMEGALY

1.6.1 GHRH testing in patients with GH-deficiency

Borges et al.(228) administered 10 µq/kq $GHRH_{1-44}$ to 12 adult patients who presented in childhood with GH-deficiency. Eight of the 12 GH-deficient patients showed no significant response, while in the remaining 4 patients the mean peak GH level after GHRH was 3.9 ± 1.2 (S.D.) ng/ml. Sm-C levels increased in 8 of 10 patients with GH-deficiency 24 hour after GHRH injec-

tion. The authors concluded that at least some patients with GH-deficiency may have hypothalamic GHRH-deficiency. Schriock et al.(194) reported a negative correlation between chronological age and peak GH responses to GHRH in children and adolescents with severe GH deficiency Furthermore peak GH levels after GHRH were reached earlier in GH-deficient children and adolescents than in GH-deficient adults. Pintor et al.(229) observed a definite increase (> 5 ng/ml) in plasma GH levels in 11 out of 14 children with isolated GH-deficiency in response to $GHRH_{1-40}$ (1 µg/kg). Gelato et al (230) reached a similar result, demonstrating a GH response to $GHRH_{1-44}$ in 17 out of 32 children with GH-deficiency (22 patients with idiopathic and 10 patients with organic GH-deficiency), which overlapped the response in age-matched normal children. Takano et al.(231,232) reported that 40% of patients with isolated GH-deficiency showed a GH response to $GHRH_{1-44}$ (1 or 2 µg/kg) greater than 5 ng/ml Pertzelan et al.(233) studied 52 patients with different forms of GH-deficiency (isolated, idiopathic multiple pituitary hormone deficiencies and organic multiple hormone deficiencies). In the group with idiopathic GH-deficiency 50% had a GH response to $GHRH_{1-44}$ (> 3 ng/ml). In the group with organic GH-deficiency only 4 out of 14 patients showed a GH response. Sixty percent of the children with isolated GH-deficiency responded to GHRH. Pintor et al.(234) observed peak GH levels ranging from 6 to 43 ng/ml in 7 subjects with idiopathic GH-deficiency. Thus, the percentage of patients with GH responses to GHRH exceeding 5 ng/ml varies in literature from 20 to 100% with an overall mean value of 50% (97). In the majority of patients a subnormal GH response is present. All these data point to a defect in GHRH secretion in most patients with GH-deficiency. It has been suggested that the longer the duration of endogenous GHRH deficiency, the less the GH response to exogenous GHRH (194). One can speculate that a somatotroph, which is unresponsive to an acute challenge of GHRH, could be stimulated by repetitive administration of GHRH in analogy to LHRH priming in hypothalamic hypogonadism. GHRH priming by 1 µg/kg given once daily subcutaneously for 5 days improved pituitary GH responsiveness in 11 out of 19 patients with GH-deficiency (235). Before priming only 7 out of 38 patients showed a GH response (> 8 ng/ml) after the first GHRH challenge. Other authors, however, reported a restoration of GH responsiveness after repetitive administration of GHRH in only 2 out of 8 children with idiopathic GH-deficiency, who did not react to the first GHRH bolus injection (236). Borges et al.(237) administered GHRH every 3 hours for 5 days at a dose of 0.33 µg/kg to six adult patients who presented in childhood with idiopathic GH-deficiency. Before priming GH levels rose after first GHRH challenge in only 2 patients, whereas after GHRH priming GH responsiveness was restored in 3 patients. It has to be noted that in this study an increase of more than 1 ng/ml was already defined as a "response". All these results in-

dicate that the somatotroph can be primed with repetitive administration of GHRH at least in some patients with GH deficiency.

1.6.2 Therapeutic use of GHRH in children with GH-deficiency

Thorner et al.(238) were the first to report acceleration of linear growth in 2 children with GH-deficiency by using $GHRH_{1-40}$ given subcutaneoulsy every 3 hours for 28 weeks in a dose of 1 or 3 µg/kg. Short term (9-12 days) iv infusion of $GHRH_{44}$ (1 µg/kg every 3 hour) resulted in an increase in lower leg growth velocity as measured by Valk's device (239) in 4 out of 7 children with GH-deficiency as compared to saline infusion (240). Similar results were reached by Smith et al.(241) administering $GHRH_{1-40}$ subcutaneously at night for four pulses at a dose of 1 µg/kg or ? µg/kg over 9 months to 5 GH-deficient children. Three patients responded to the treatment with an increase of growth velocity. One patient showed a modest increase in growth velocity with the higher dose. One subject did not react to the treatment. The group of Besser treated 18 prepubertal GH-deficient children with twice daily subcutaneous injections of $GHRH_{1-29}$ (242). Twelve of the children showed a good response to GHRH. The remaining 6 had a modest increase in height velocity. Further studies are needed to establish the optimum dose and mode of administration of GHRH in GH deficient children. The results of the "GRF European Multicenter Study" have to be awaited.

1.6.3 GHRH testing in short children

Takano et al.(231,232) administered an acute bolus injection (iv) of 1 and 2 µg/kg body weight to 139 normal children with short stature (height below 2 S.D. of the mean height of Japanese boys and girls). Peak GH levels after 1 and 2 µg/kg GHRH were reached at 15 and 30 minutes respectively (32 ± 4 and 32 + 2 ng/ml). Peak GH levels were greater after GHRH than after insulin-induced hypoglycemia. Similar results were reported by other authors (195,233). Peak GH levels achieved after GHRH were not different from those in normal adults. However, acute GHRH testing cannot exclude disturbances in GH secretion in short children, as peak GH levels after GHRH testing poorly correlated with integrated 24 h mean GH levels in GH-deficient and short normal children and in children with growth hormone neurosecretory dysfunction (243). The latter group was defined by a mean serum 24 h GH concentration below 3 ng/ml and a normal response (> 10 ng/ml) to classical GH provocative tests (243,244). Therefore the GHRH test cannot replace the classical GH provocative tests in the diagnostic work-up of children with short stature or delay in growth.

Pretreatment of normal and short normal children with propranolol aug-

mented the GHRH-induced GH release in all subjects (217). As mentioned earlier (par. 1.5.4.) this effect might be mediated by inhibition of SRIF secretion. In children with constitutional growth delay oxandrolone, an anabolic steroid, enhanced the GH secretion in response to GHRH (245).

1.6.4 Use of GHRH in patients with diabetes mellitus

Press et al.(246) administered 0.3 µg/kg $GHRH_{1-40}$ to 12 poorly controlled type 1 diabetics and healthy control subjects. Although basal GH levels were elevated in the diabetics (7.1 ± 1.8 ng/ml), peak GH responses after GHRH were similar in diabetics and normal subjects (30 + 5 ng/ml). A group of well controlled type 1 diabetics (with insulin pump) showed normal basal GH levels and peak GH responses not different from the other 2 groups. The normal subjects had a marked suppression of the response to GHRH after glucose-loading. Thus, insufficient GH suppression of the pituitary in response to hyperglycemia exists in poorly controlled type 1 diabetes, thereby probably contributing to this poor metabolic control. Similar results were reported by Sharp et al.(247). Kaneko et al.(248) demonstrated that GH responses to GHRH were more pronounced in type 1 diabetics with retinopathy than in diabetics without this complication. The 2 groups did not differ in biochemical characteristics. The authors have no explanation for their findings.

1.6.5 Studies with GHRH in morbid obesity

Administration of $GHRH_{1-40}$ (1 µg/kg) to 10 morbidly obese patients resulted in markedly impaired GH responses as compared to normal weight controls (249). After achieving a normal weight, GH responsiveness to GHRH partially restored. A negative correlation was found between the GH response to GHRH and the percentage of ideal body weight. The reversibility of the defect in GH secretion suggests that it is a consequence rather than a cause of obesity. Similar results were reported by other authors (250-253).

1.6.6 Influence of thyroid status on GH responsiveness to GHRH

In hypothyroidism a significant reduction in peak GH response and integrated GH levels in response to GHRH is observed, compared to those in the euthyroid state (254). These results are in agreement with in vitro and in vivo observations in animals (see before) and were confirmed by other authors (255). However, in 3 out of 14 patients GH responsiveness to GHRH only partially restored after 6 weeks of thyroxine replacement, despite reduction of TSH levels. Further studies are needed to more precisely de-

lineate when GH responsiveness to GHRH in hypothyroidism after starting thyroxine replacement is restored.

1.7 CLINICAL STUDIES WITH GHRH IN PATIENTS WITH ACROMEGALY

1 7 1 Pathophysiology of acromegaly

The first documented case of acromegaly probably was the Egyptian pharao Akhnaton (256). The king had prognathism, enlargement of the nose and upper jaw and thickening of the face and lips, but normal sized hands and feet. Therefore the diagnosis of acromegaly is not entirely certain. Pierre Marie (257) was the first to describe the clinical features of acromegaly. Later it was found that the disease was associated with an enlargement of the pituitary (258). Cushing (259) was one of the first to demonstrate that the features of acromegaly were reversible after pituitary surgery (using the transsphenoidal approach). A role of a growth promoting hormone produced by the pituitary was postulated since 1921 (260) as causative in the etiology of acromegaly. Confirmation of this hypothesis was possible after assays of GH became available (261). Pituitary overproduction of GH could now be established as the cause of acromegaly.

Pituitary adenomas are present in 15 to 25% of all subjects at autopsy (262), 1% of which secreted GH (256).

Very recently a new pathological classification of pituitary adenomas has been introduced by Melmed et al.(256,263). Tumors are classified according to their GH content, their ultrastructural features and cytogenesis. These authors classified the pituitary of patients with acromegaly in 9 different categories.

- The pure GH producing adenomas may either be densely granulated or sparsely granulated. The densely granulated adenomas contain large amounts of stored GH and grow slowly and have an insidious clinical course. They occur in 30% of acromegaly.
- In contrast the sparsely granulated adenomas grow fast, locally invasive and often grow suprasellarly. Thirty procent of all GH producing adenomas belong to this category.
- Adenomas with a mixture of GH-producing and prolactin-producing cells compromise 25% of all adenomas. Usually they are associated with mild to severe acromegaly and elevated prolactin levels.
- Acidophilic stem cell adenomas (10%) are composed of immature cells, which are believed to be the progenitor cells of both the GH and prolactin producing cell. They grow rapidly and often locally invasive. Only one cell type is found which produces both GH and prolactin.
- The mammasomatotroph adenomas (4%) are composed of one cell type,

supposed to be a more mature variant of the acidophil stem cell The same cell can simultaneously contain GH and prolactin The clinical course is usually mild.

- The unclassified plurihormonal adenomas are also rare (6%) They are monomorph or plurimorph and can produce besides GH and prolactin other pituitary hormones predominantly TSH and ACTH. Accordingly the clinical spectrum of these adenomas is variable.
- GH cell carcinomas are extremely rare They can give distant metastases.
- GH cell hyperplasia is a morphological difficult diagnosis, which is often associated with ectopic or eutopic GHRH producing tumors The latter are hypothalamic tumors, such as hamartomas and gangliocytomas (54). By immunocytochemical staining GHRH has been localized in these tumors (54). The excessive GHRH production causes GH-cell hyperplasia. Ectopic, extrapituitary production of GHRH by carcinoid tumors, lung adenocarcinomas and other tumors has been discussed in the first section of this introduction.
- Rarely, no pituitary abnormality at all is found in patients with acromegaly.

Until now the pathogenesis of acromegaly remains obscure Hypotheses have been put forward which state that the pituitary adenoma originates either from a primary pituitary defect of GH producing cells or from a hypothalamic dysregulation (256,264,265). According to the latter hypothesis overproduction of GH can result from GHRH excess or defective secretion of SRIF. When endogenous SRIF deficiency is the cause of acromegaly one might expect that the GH overproduction can be corrected by exogenous SRIF infusion. Endogenous GHRH excess, with normal or elevated endogenous SRIF secretion should theoretically be less sensitive to exogenous SRIF infusion. Hanew et al.(266) demonstrated that the higher SRIF responsiveness was found in those acromegalics whose GH levels anomalously rose after TRH or LHRH administration, not, however, in TRH or LHRH non-responders. Similar results were reported by Pieters et al.(265) showing that the subgroup of acromegalics, whose GH levels normalized after SRIF infusion, had a paradoxical GH increase after LHRH. These data and those of Hanew et al. (266) suggest that at least in some acromegalics endogenous SRIF deficiency and/or SRIF resistance may contribute to the pathogenesis of the disease. We found a wide spectrum of sensitivity to the suppressive action of SRIF on basal and GHRH stimulated GH secretion in acromegalics. SRIF induced GH suppression was more pronounced in the patients with the lower basal GH levels, whereas inadequate suppression was observed in those with the highest levels (this thesis). Very recently Kelijman et al.(267) adduced similar evidence for this thesis, in studies both in vivo and in

vitro SRIF resistance thus may reflect an intrinsic abnormality of the neoplastic somatotroph

Other factors which favour the hypothesis that acromegaly is caused by a hypothalamic or central defect in GH modulation will be briefly discussed (256). The fact that sometimes a normal pituitary histology is found in patients with acromegaly (268,269) points to a hypothalamic or central origin After surgical removal of a GH secreting adenoma, the GH responses to provocative stimuli often remain abnormal despite normalization of basal GH levels (264). These data also suggest that there is an abnormality in the control of GH regulation. As most acromegalics respond to GHRH with a rise in GH secretion (81,82,270, this thesis, Chapter 4.4), the pituitary adenomas in these patients are not functioning completely autonomously. Older observations that the GH responses to GH-provocative stimuli, which act by central mechanisms (insulin, arginine, exercise) are comparable in acromegalics and normal subjects (271,272) also point to the persistence of hypothalamic control of GH secretion in acromegalics. However, the fact that the suppression of GH secretion found in normal subjects after glucose loading is usually absent in acromegaly or that glucose loading even gives rise to a paradoxical GH increase is also evidence in favour of hypothalamic dysregulation. The paradoxical decrease of GH secretion after dopaminergic agents (such as bromocriptine) in more than 50% of acromegalics (273) in contrast to their GH-increasing effect in normal subjects (215,274) also might reflect a disturbance in the influence of hypothalamic or higher structures on GH secretion in acromegaly. Another possibility, however, is a change in the dopaminergic receptors on the adenomatous somatotrophs (264). Other authors suggested a possible pathogenetic role of an increased serotoninergic tone in acromegaly (275). The rare cases of GHRH secreting hypothalamic hamartomas, as discussed previously, are a direct hypothalamic cause of acromegaly.

There is also, however, a lot of evidence suggesting that acromegaly is a primarily pituitary disease. The finding of normal pituitary tissue surrounding a GH-secreting adenoma favours the concept that the adenoma is formed autonomously in the pituitary. The restoration of normal GH dynamics following pituitary surgery for acromegaly is said to be evidence in favour of a primary pituitary genesis of acromegaly (256). Persistence of abnormal GH responses to provocative stimuli (TRH or LHRH) after pituitary surgery can, however, also be interpreted as a permanent hypothalamic dysregulation in GH secretion (vide supra). Alternatively, residual adenomatous tissues may be responsible for a persistent anomalous response. The fact that the abnormal and paradoxical GH responses to dopaminergic agents and/or nonspecific releasing hormones are also found in cultured GH-secreting adenomas in vitro (276,277) lends support to the hypothesis of a pri-

marily pituitary genesis of acromegaly. The abnormal GH responses to the fore-mentioned agents could be caused by the presence of receptors on the adenomatous tissues not expressed on normal somatotrophs. It is not clear whether these receptors are influenced or induced by hypothalamic factors, as might be the case in the paradoxical GH increments after TRH in various conditions as diabetes mellitus (278), anorexia nervosa (279), renal failure (280), chronic liver disease (281), primary hypothyroidism (282) and mental depression (283), clearly situations where no pituitary adenoma exists.

Taken together there are arguments favouring a hypothalamic genesis of acromegaly, whereas other are more indicative of a pituitary origine of acromegaly. Combining the different hypotheses one could speculate that intrinsically abnormal somatotrophs in the pituitary could be transformed to autonomous adenomas under the influence of hypothalamic factors such as continuous stimulation by GHRH and/or diminished inhibition by SRIF (264).

1.7.2 Studies with GHRH in acromegaly

It was hoped that the availability of GHRH could afford new insights in the pathogenesis of acromegaly. Wood et al.(284) were among the first to administer a bolus injection of 100 µg $GHRH_{1-44}$ to six acromegalics. The acromegalics could be divided in two groups, those with a GH response to GHRH not different from that in control subjects and whose GH levels significantly suppressed after oral glucose, and those acromegalics with an exaggerated GH response to GHRH, whose GH levels did not suppress after oral glucose loading. Gelato et al. (285) reported a tendency for the GH response to GHRH to increase with increasing suppression to glucose, although the data lacked statistical significance. Losa et al.(286) did not find any correlation between the outcome of the GHRH test in acromegalics and other dynamic tests of GH secretion such as oral glucose loading. A number of authors (284-289) demonstrated a highly variable GH response to administration of an acute bolus injection of GHRH to acromegalics, which partially overlapped the responses seen in normal subjects. In a study reported in this thesis Pieters et al. (Chapter 4.4) were the first to suggest that the large variability in the GH responses to GHRH was the consequence of the highly variable basal GH values, as they could calculate a highly significant close correlation between both.

It has been suggested that GHRH does not stimulate GH release in acromegalics with the ectopic GHRH syndrome (53,287,290). However Barkan et al.(291) discussed an acromegalic patient with a GHRH producing carcinoid tumor who had a preserved acute GH response to exogenous GHRH. Therefore no pattern of GH secretion is diagnostic of acromegaly due to ectopic GHRH production (53). The only way to differentiate patients with acromegaly

due to ectopic GHRH production from acromegalics with a pituitary adenoma is by measuring plasma GHRH levels (196,292). Thorner et al.(292) measured plasma GHRH levels in 177 acromegalics. GHRH levels were maximally 62.5 ng/l in normal subjects and 82 ng/l in the acromegalics. Thus there was a huge overlap in GHRH levels between normal subjects and patients with acromegaly. Three patients with known GHRH secreting tumors had GHRH levels, ranging from 2000 - 2440 ng/l. GHRH levels were measured using three different assays. Penny et al.(196) found immunoreactive GHRH levels in normal subjects ranging from < 10 to 60 ng/l, which completely overlapped the values found in 76 out of 80 acromegalics. The remaining 4 patients had values ranging from 92 to 25000 ng/l. The highest value was found in the only one patient with proven ectopic GHRH syndrome.

Pieters et al. (Chapter 4.4) and Arosio et al.(293) investigated the effects of SRIF infusion on concomitant GHRH injection in patients with acromegaly. SRIF significantly suppressed GH levels in all patients and blunted the GH response to GHRH. The GH answer to GHRH was highly variable. The authors suggested that the different GH responses to GHRH might reflect a different sensitivity of the adenomatous somatotrophs and a possible contribution of the normal somatotrophs to the GHRH-induced GH secretion. No correlations were found between GH responsiveness to GHRH and the abnormal GH responses to TRH and dopamine infusion.

Losa et al.(294) reported that GHRH induces prolactin secretion in acromegalics, but not in normal subjects. Most authors, however, do not give data on prolactin responsiveness to GHRH.

Cozzi et al.(295) administered GHRH and TRH to 24 acromegalics before and during chronic bromocriptine (Br) treatment. Br did not alter GH responsiveness to GHRH, but reduced the GH response to TRH. It appeared that the initial GH response to GHRH was significantly lower in the Br nonresponders (i.e. mean daily GH reduction during Br treatment less than 50%). In contrast the GH response to TRH was most pronounced in the Br responders. From these data the authors suggested that in GH-secreting pituitary adenomas, cells responsive to Br and TRH (lactotroph-like) and cells sensitive to GHRH (somatotroph-like) may coexist. Chiodini et al.(289) found an inverse correlation between the percentage of GH changes after GHRH and Br. No relationship was found between the GH responses to GHRH and to TRH. These data can be explained by a similar way of reasoning. Other authors, however, failed to find specific combinations of GH responses to GHRH, TRH, Br and LHRH (285,293,296,297).

Losa et al.(298) administered a 50 μg bolus injection of $GHRH_{44}$ to 9 acromegalic patients, followed by a 2-hour infusion of GHRH (100 μg/h), after which a second bolus injection of 50 μg GHRH was given and compared the results with those obtained in normal subjects. Normal subjects showed a steady decline of the initially increased GH levels, despite continua-

tion of the GHRH infusion. In contrast, the GHRH-stimulated GH levels in the acromegalics did not decrease during the infusion. This difference could not be explained by differences in GHRH levels between the control subjects and the acromegalics. The authors hypothesized that the sustained elevation of GH levels in the acromegalics could be explained by a greater readily releasable GH pool and/or faster GH synthesis in the patients as compared to the controls. An alternative explanation might be that continuous infusion of GHRH in normal subjects leads to desensitization (vide supra), which phenomenon is absent in acromegalics. The fact that in normal subjects GH responses to a GHRH bolus injection after continuous infusion of GHRH is inversely related to the amount of GH released during the infusion of GHRH (299) points to a limited pool of readily releasable GH. A larger releasable GH pool in acromegalics is also compatible with the observation that GH responsiveness to GHRH in acromegalics is positively correlated with the basal GH level (286, Chapter 4.4). Shibasaki et al.(297) infused acromegalics and control subjects with 1 mg GHRH during 150 minutes. At the end of the infusion a bolus injection of GHRH was given. The infusion of GHRH caused a sustained increase in GH levels both in the normal subjects and acromegalics. The subsequent bolus injection of GHRH at the end of the infusion, however, did not further increase GH levels in both groups. This latter observation is in agreement with the data of Losa et al.(298). The authors explained the data by suggesting that desensitization occurs in normal subjects as well as in acromegalics

Repetitive administration of GHRH (50 μg every 2 hours three times caused a blunting of the GH response after the second and third GHRH bolus in control subjects, but not in acromegalic patients (300). The larger readily releasable GH pool and/or faster GH turnover in the adenomatous somatotrophs was held responsible for the observed difference. A similar study design was used by Spada et al.(301) to investigate whether desensitization occurs in acromegaly after continuous or repetitive administration of GHRH, in vivo and in vitro. In normal subjects a marked elevation of GH levels was only observed after the first bolus GHRH, not after the second and third injection. In the acromegalics, however, the second and third injection of GHRH resulted in GH responses quite similar to those after the first one. In vitro, GHRH pretreatment of GH secreting adenomas in monolayer culture did not lead to a diminished GH response after an acute GHRH challenge. From these data the authors concluded that in acromegaly GH responsiveness to GHRH persists, despite continuous presence of GHRH, in vivo as well as in vitro. This phenomenon can at least in part be explained by assuming a primary impairment of the desensitization process in acromegalics. However, the finding of elevated GH levels in acromegalics with ectopic GHRH production does not favour this hypothesis.

Nakagawa et al (302) studied the effect of dexamethasone (9 mg/day for 2 days) on the GH response to GHRH in acromegaly Dexamethasone decreased GH responsiveness to GHRH in vivo In vitro, however, dexamethasone pretreatment potentiated the GH response to GHRH on monolayer cultures of pituitary adenomas of three of these patients. These results indicate that the GH potentiating effects of dexamethasone in vitro may be overwhelmed by other influences possibly induced by dexamethasone in vivo. Ceda et al.(63), however, observed a suppression of GHRH mediated GH release after 4 hr of dexamethasone incubation of human pituitary tumor cells from an acromegalic patient.

Pietschmann et al.(303) studied the GH response to GHRH before and after treatment with the cholinergic muscarinic receptor blocker atropine in acromegalics, patients with type I diabetes mellitus and normal subjects Atropine pretreatment virtually completely blocked the GH response to GHRH in the diabetics and normal subjects, but did not suppress the GH answer to GHRH in the acromegalic patients From these data the authors concluded that acromegalics have a defect in the cholinergic control of GH secretion.

In an elegant study Hanew et al.(304) investigated the effects of dopamine (DA) on GH secretion in patients with acromegaly. The authors compared the GH-lowering effect of DA, which does not cross the blood-brain barrier, with that of Br and L-Dopa (a precursor of DA), which both pass the blood-brain barrier. Furthermore the effect of combined administration of L-Dopa and domperidone (a peripheral dopamine-antagonist which does not cross the blood-brain barrier and blocks the DA receptors in the pituitary) on GH secretion in acromegaly was studied. DA, L-Dopa and Br lowered GH levels in all the patients with the greatest decrease occurring after DA. Domperidone alone had no effect on GH levels. The combination of domperidone and L-Dopa, however, significantly increased GH levels. These data suggest that in acromegaly DA not only directly inhibits GH secretion in the pituitary but also indirectly stimulates this secretion via the hypothalamus. Probably DA acts by a central stimulating effect on GHRH release, although an inhibiting effect on SRIF release could not totally be excluded. The authors further suggested that there might be a defect in the GH negative feedback between pituitary and hypothalamus because the dopaminergic hypothalamic GH-releasing mechanism expectedly would be suppressed by the excessive GH secretion, which is obviously not the case.

Taken together availability of GHRH has elucidated important aspects in the pathogenesis of acromegaly, although as yet no universally acceptable theory has emerged.

The discovery of GHRH as another hypothalamic releasing hormone provided a new tool to unravel the complex regulation of growth hormone production

and secretion. Together with somatostatin GHRH enables fine tuning of these processes at the hypophyseal level. Moreover, immunohistochemical studies with the use of GHRH-antibodies enables to get some insight in the complex interaction of growth hormone, somatostatin and GHRH at the hypothalamic level. In recent years there is growing consensus that besides short loop feedback between hypophysis and hypothalamus, intra-hypothalamic ultra-short feedback contributes to regulation of hypothalamic hypophyseal physiological functioning.

In pathology, the availability of GHRH for testing hypophyseal function has opened new vistas on the causes of growth disorders. Besides finding defects of GH synthesis and/or release in the hypophysis one has realized that also hypothalamic damage with resulting GHRH deficiency and/or uncontrolled production of somatostatin may be a cause of growth hormone deficiency. Treatment of some forms of these disorders with GHRH has become a new and intriguing possibility as has been shortly reviewed in paragraph 1.6.2 of this chapter. The study of GHRH-growth hormone relations in acromegaly may lead to better understanding of the role of hypothalamic and hypophyseal pathology in the causation of this disorder.

REFERENCES

1. Green JD, Harris GW (1947) The neurovascular link between the neurohypophysis and adenohypophysis. J Endocrinol, 5: 136.
2. Reichlin S (1960) Growth and the hypothalamus. Endocrinol, 67: 760.
3. Deuben RR, Meites J (1964) Stimulation of pituitary growth hormone release by a hypothalamic extract in vitro. Endocrinol 74: 408.
4. Frohman LA, Bernardis LL (1968) Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. Endocrinol 82: 1125.
5. Frohman LA, Bernardis LL, Kant KJ (1969) Hypothalamic stimulation of growth hormone secretion. Science 162: 580.
6. Frohman LA, Maran JW, Dhariwal APS (1971) Plasma growth hormone responses to intrapituitary injections of growth hormone releasing factor (GFR) in the rat. Endocrinol 88: 1483.
7. Johanssen KNG, Currie BL, Folkers K (1974) Identification and purification of factor B-GHRH from hypothalami which releases growth hormone. Biochem Biophys Res Commun 60: 610.
8. Takahara J, Arimura A, Schally AV (1975) Assessment of GH releasing hormone activity in Sephadex-separated fractions of porcine hypothalamic extracts by hypophyseal portal vessel infusions in the rat. Acta Endocrinol (Copenh) 78: 424.
9. Krulich L, Dhariwal APS, McCann SM (1968) Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinol 83: 783.
10. Brazeau P, Vale W, Burgus R, Ling L, Butcher M, Rivier J, Guillemin R (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179: 77.

11. Southern AL (1960) Functioning metastatic bronchial carcinoid with elevated levels of serum and cerebrospinal fluid serotonin and pituitary adenoma. J Clin Endocrinol Metab 20: 298.
12. Buse J, Buse M, Roberts WJ (1961) Eosinophilic adenoma of the pituitary and carcinoid tumor of the rectosigmoid area. J Clin Endocrinol Metab 21· 735.
13. Weiss L, Ingram M (1961) Adenomatoid bronchial tumors· a consideration of the carcinoid tumors and the salivary tumors of the bronchial tree. Cancer 14: 161.
14. Sonksen PH, Ayres AB, Brainbridge M et al.(1976) Acromegaly caused by pulmonary carcinoid tumours. Clin Endocrinol 5· 503.
15. Shalet SM, Beardwell CG, MacFarlane IA, Ellison ML, Norman CM, Rees LH, Hughes M (1979) Acromegaly due to production of a growth hormone releasing factor by a bronchial carcinoid tumour. Clin Endocrinol 10· 61.
16. Frohman LA, Szabo M, Berelowitz M, Stachura ME (1980) Partial purification and characterization of a peptide with growth hormone-releasing activity from extrapituitary tumors in patients with acromegaly. J Clin Invest 65: 43.
17. Thorner MO, Perryman RL, Cronin MJ, Rogol AD, Draznin M, Johanson A, Vale W, Horvath E, Kovacs K (1982) Somatotroph hyperplasia. Successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. J Clin Invest 70: 965.
18. Rivier J, Spiess J, Thorner M, Vale W (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature 300: 276.
19. Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science 218: 585.
20. Esch FS, Bohlen P, Ling NC, Brazeau PE, Wehrenberg WB, Thorner MO, Cronin MJ, Guillemin R (1982) Characterization of a 40 residue peptide from a human pancreatic tumor with growth hormone releasing activity. Biochem Biophys Res Comm 109· 152.
21. Sassolas G, Chayvialle JA, Partensky C, Berger G, Thouillas J, Berger F, Claustrat B, Cohen R, Girod C, Guillemin R (1983) Acromégalie, expression clinique de la production de facteurs de libération de l'hormone de croissance (GRF) par une tumeur pancréatique. Ann d'Endocrinol Paris 44: 347.
22. Bohlen P, Brazeau P, Bloch B, Ling N, Gaillard R, Guillemin R (1983) Human hypothalamic growth hormone releasing factor (GRF)· evidence for two forms identical to tumor derived GRF-44-NH_2 and GRF-40. Biochem Biophys Res Comm 114: 930.
23. Ling N, Esch F, Bohlen P, Brazeau P, Wehrenberg WB, Guillemin R (1984). Isolation, primary structure, and synthesis of human hypothalamic somatocrinin: growth hormone-releasing factor. Proc Natl Acad Sci USA 81· 4302.
24. Spiess J, Rivier J, Vale W (1983) Characterization of rat hypothalamic growth hormone-releasing factor. Nature 303: 532.
25. Bohlen P, Esch F, Brazeau P, Ling N, Guillemin R (1983) Isolation and characterization of the porcine hypothalamic growth hormone releasing factor. Biochem Biophys Res Comm 116: 726.
26. Esch F, Bohlen P, Ling N, Brazeau P, Guillemin R (1983) Isolation and characterization of the bovine hypothalamic growth hormone releasing factor. Biochem Biophys Res Comm 117· 772.

27. Brazeau P, Böhlen P, Esch FS, Ling N, Wehrenberg WB, Guillemin R (1984) Growth hormone-releasing factor from ovine and caprine hypothalamus: isolation, sequence analysis and total synthesis. Biochem Biophys Res Comm 125: 606.
28. Gubler U, Monahan JJ, Lomedico PT, Bhatt RS, Collier KJ, Hoffman BJ, Bohlen P, Esch F, Ling N, Zeytin F, Brazeau P, Poonian MS, Gage LP (1983) Cloning and sequence analysis of cDNA for the precursor of human growth hormone-releasing factor, somatocrinin. Proc Natl Acad Sci USA 80: 4311.
29. Mayo KE, Cerelli GM, Lebo RV, Bruce BD, Rosenfeld MG. Evans RM (1985) Gene encoding human growth hormone-releasing factor precursor: structure, sequence, and chromosomal assignment. Proc Natl Acad Sci USA 82: 63.
30. Bloch B, Gaillard RC, Brazeau P, Lin HD, Ling N (1984) Topographical and ontogenetic study of the neurons producing growth hormone-releasing factor in human hypothalamus. Reg Peptides 8: 21.
31. Bresson JL, Clavequin MC, Fellmann D, Bugnon L (1984) Ontogeny of the neuroglandular system, revealed with HpGRF44 antibodies in human hypothalamus. Neuroendocrinol 39: 68.
32. Pelletier G, Désy L, Côté J, Lefèvre G, Vaudry H (1986) Light-microscopic immunocytochemical localization of growth hormone-releasing factor in the human hypothalamus. Cell Tissue Res 245: 461.
33. Werner H, Okon E, Fridkin M, Koch Y (1986) Localization of growth hormone-releasing hormone in the human hypothalamus and pituitary stalk. J Clin Endocrinol Metab 63: 47.
34. Jacobowitz DM, Schulte H, Chrousos GR, Loriaux DL (1983) Localization of GRF-like immunoreactive neurons in the rat brain. Peptides 4: 521.
35. Sawchenko PE, Swanson LW, Rivier J, Vale W (1985) The distribution of growth-hormone-releasing factor (GRF) immunoreactivity in the central nervous system of the rat: An immunohistochemical study using antisera directed against rat hypothalamic GRF. J Comp Neurol 237: 100.
36. Smith RM, Howe PRC, Oliver JR, Willoughby JO (1984) Growth hormone releasing factor immunoreactivity in rat hypothalamus. Neuropept 4: 109.
37. Fellmann D, Bugnon C, Lavry GN (1985) Immunohistochemical demonstration of a new neurone system in rat brain using antibodies against human growth hormone-releasing factor (1-37). Neurosc Lett 58: 91.
38. Everitt BJ, Meister B, Hökfelt T, Melander T, Terenius L, Rökaeus A, Theodorsson-Norheim E, Dockray G, Edwardson J, Cuello C, Elde R, Goldstein M, Hemmings H, Quimet C, Waklaas I, Greengard P, Vale W, Weber E, Wu J, Chang K (1986) The hypothalamic arcuate nucleus-median eminence complex: immunohistochemistry of transmitters, peptides and DARPP-32 with special reference to coexistence in dopamine neurons. Brain Res Rev 11: 87.
39. Van de Pol CJ, Leidy JW, Finger TE, Robbins RJ (1986) Immunohistochemical localization of GRF-containing neurons in rat brain. Neuroendocrinol 42: 143.
40. Bloch B, Brazeau P, Ling N, Böhlen P, Esch F, Wehrenberg WB, Benoit R, Bloom F, Guillemin R (1983) Immunohistochemical detection of growth hormone-releasing factor in brain. Nature 301: 607.
41. Lechan RM, Lin HD, Ling N, Jackson IMD, Jacobson S, Reichlin S (1984) Distribution of immunoreactive growth hormone releasing factor (1-44) NH_2 in the tuberoinfundibular system of the rhesus monkey. Brain Res 309: 55.
42. Morel G, Mesquich P, Dubois MP, Dubois PM (1984) Ultrastructural evi-

dence for endogenous growth hormone-releasing factor-like immunoreactivity in the monkey pituitary gland. Neuroendocrinol 38: 123.
43. Girod C, Trouillas J (1985) Immunocytochemical localization of growth hormone-releasing factor in the monkey (Macaca irus) hypothalamus and median eminence. Biomed Res 6: 153.
44. Bugnon C, Gouget A, Fellmann D, Clavequin MC (1983) Immunocytochemical demonstration of a novel peptidergic neurone system in the cat brain with an anti-growth hormone-releasing factor serum. Neurosc Lett 38: 131.
45. Andries JC, Belemtougri G, Tramu G (1984) Immunohistochemical identification of growth hormone-releasing factor-like material in the nervous sytem of an insect, aeshua cyanea (odonata). Neuropept 4: 519.
46. Bosman FG, van Assche C, Kruseman ACN, Jackson S, Lowry PJ (1984) Growth hormone releasing factor (GRF) immunoreactivity in human and rat gastrointestinal tract and pancreas. J Histochem Cytochem 32: 1139.
47. Christotides ND, Stephanon A, Suzuki H, Yiangou Y, Bloom SR (1984) Distribution of immunoreactive growth hormone-releasing hormone in the human brain and intestine and its production by tumors. J Clin Endocrinol Metab 59: 747.
48. Shibasaki T, Kiyosawa Y, Masuda A, Nakahara M, Imaki T, Wakabayashi I, Demura H, Shizume K, Ling N (1984) Distribution of growth hormone-releasing hormone-like immunoreactivity in human tissue extracts. J Clin Endocrinol Metab 59: 263.
49. Tanaka Y, Saito S, Namba I, Ohasi S, Sawano S, Irie M (1984) Study on human pancreatic growth hormone releasing factor-like substance in human placenta. 7th Int Congr Endocrinol, Quebec, Abstr 2335.
50. Kashio Y, Chihara K, Kita T, Okimura Y, Sato M, Kadowaki S, Fujita T (1987) Effect of oral glucose administration on plasma growth hormone-releasing hormone (GHRH)-like immunoreactivity levels in normal subjects and patients with idiopathic GH deficiency: evidence that GHRH is released not only from the hypothalamus but also from extrahypothalamic tissue. J Clin Endocrinol Metab 64: 92.
51. Roth KA, Wilson DM, Eberwine J, Dorin RI, Kovacs K, Bensch KG, Hoffman AR (1986) Acromegaly and pheochromocytoma: a multiple endocrine syndrome caused by a plurihormonal adrenal medullary tumor. J Clin Endocrinol Metab 63: 1421.
52. Asa SL, Kovacs K, Thorner MO, Leong DA, Rivier J, Vale W (1985) Immunohistological localization of growth hormone-releasing hormone in human tumors. J Clin Endocrinol Metab 60: 423.
53. Boizel R, Halimi S, Labat F, Cohen R, Bachelot J (1987) Acromegaly due to a growth hormone-releasing hormone-secreting bronchial carcinoid tumor: Further information on the abnormal responsiveness of the somatotroph cells and their recovery after successful treatment. J Clin Endocrinol Metab 64: 304.
54. Asa S, Scheithauer BW, Bilbao JM, Horvath E, Ryan N, Kovacs K, Randall RV, Laws ER jr, Singer W, Linfoot JA, Thorner MO, Vale W (1984) A case of hypothalamic acromegaly: A clinicopathological study of six patients with hypothalamic ganglliocytomas producing growth hormone-releasing factor. J Clin Endocrinol Metab 58: 796.
55. Meister B, Hökfelt T, Vale W, Goldstein M (1985) Growth hormone releasing factor (GRF) and dopamine coexist in hypothalamic arcuate neurons. Acta Physiol Scand 124: 133.
56. Meister B, Hökfelt T, Vale W, Sawchenko PE, Swanson L, Goldstein M

(1986) Coexistence of tyrosine hydroxylase and growth hormone-releasing factor in a subpopulation of tubero-infundibular neurons of the rat. Neuroendocrinol 42: 237.
57. Okamura H, Murakami S, Chihara K, Hagatsu I, Ibata Y (1985) Coexistence of growth hormone releasing factor-like and tyroxine hydroxylase-like immunoreactivities in neurons of the rat arcuate nucleus. Neuroendocrinol 41: 177.
58. Fellmann D, Bugnon C, Verstegen J, Lavry GN (1986) Coexistence of human growth hormone-releasing factor 1-37-like and α-melanotropin-like immunoreactivities in neurones of the rat lateral dorsal hypothalamus. Neurosc Lett 68: 122.
59. Fuxe K, Andersson K, Harfstrand A, Agnati LF, Eneroth P, Janson AM, Vale W, Thorner M, Goldstein M (1986) Medianosomes as integrative units in the external layer of the median eminence. Studies on GRF/catecholamine and somatostatin/catecholamine interactions in the hypothalamus of the male rat. Neurochem Int 9: 155.
60. Veliçelebi G, Santacroce TM, Harpold MM (1985) Specific binding of synthetic human pancreatic growth hormone releasing factor (1-40-OH) to bovine anterior pituitaries. Biochem Biophys Res Comm 126: 33.
61. Seifert H, Perrin M, Rivier J, Vale W (1985) Growth hormone-releasing factor binding sites in rat anterior pituitary membrane homogenates: modulation by glucocorticocoids. Endocrinol 117: 424.
62. Seifert H, Perrin M, Rivier J, Vale W (1985) Binding sites for growth hormone releasing factor on rat anterior pituitary cells. Nature 313: 487.
63. Ceda GP, Davis RG, Hoffman AR (1987) Glucocorticoid modulation of growth hormone secretion in vitro. Evidence for a biphasic effect on GH-releasing hormone mediated release. Acta Endocrinol (Copenh) 114: 465.
64. Dieguez C, Foord SM, Peters JR, Hall R, Scanlon MF (1985) The effects of thyroid hormone deprivation in vivo and in vitro on growth hormone (GH) responses to human pancreatic (Tumor) GH-releasing factor (1-40) by dispersed rat anterior pituitary cells. Endocrinol 116: 1066.
65. Brazeau P, Ling N, Bohlen P, Esch F, Ying S, Guillemin R (1982) Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone in vitro. Proc Natl Acad Sci USA 79: 7909.
66. Brazeau P, Ling N, Esch F, Bohlen P, Monqin C, Guillemin R (1982) Somatocrinin (Growth hormone releasing factor) in vitro bioactivity; Ca^{++} involvement, cAMP mediated action and additivity of effect with PGE_2. Biochem Biophys Res Comm 109: 588.
67. Cronin MJ, Roqol AD, Dabney LG, Thorner MO (1982) Selective growth hormone and cyclic-amp stimulating activity is present in a human pancreatic islet cell tumor. J Clin Endocrinol Metab 55: 381.
68. Bilezikjian LM, Vale WW (1983) Stimulation of adenosine 3',5'-monophosphate production by growth hormone-releasing factor and its inhibition by somatostatin in anterior pituitary cells in vitro. Endocrinol 113: 1726.
69. Canonico PL, Cronin MJ, Thorner MO, MacLeod RM (1983) Human pancreatic GRF stimulates phosphatidylinositol labeling in cultured anterior pituitary cells. Am J Physiol 245: E 587.
70. Cronin MJ, Roqol AD, Macleod RM, Keefer DA, Loqin IS, Borges JLC, Thorner MO (1983) Biological activity of a growth hormone-releasing factor secreted by a human tumor. Am J Physiol 244: E346.
71. Michel D, Lefèvre G, Labrie F (1983) Interactions between growth

hormone-releasing factor, prostaglandin E_2 and somatostatin on cyclic AMP accumulation in rat adenohypophyseal cells in culture. Mol Cell Endocrinol 33: 255.
72. Vale W, Vaughan J, Yamamoto G, Spiess J, Rivier J (1983) Effects of synthetic human pancreatic (tumor) GH releasing factor and somatostatin, triiodothyronine and dexamethasone on GH secretion in vitro. Endocrinol 112: 1553.
73. Fink G, Jamieson MG (1976) Immunoreactive luteinizing hormone releasing factor in rat pituitary stalk blood: effects of electrical stimulation of the medial preoptic area. J Endocrinol 68: 71.
74. Neill JD, Patton JM, Dailey RA, Tsou RC, Tindall GT (1977) Luteinizing hormone releasing hormone (LHRH) in pituitary stalk blood of rhesus monkeys: relationship to level of LH release. Endocrinol 101: 430.
75. Chihara K, Arimura A, Schally AV (1979) Immunoreactive somatostatin in rat hypophyseal portal blood: effects of anesthetics. Endocrinol 104: 1434.
76. Arimura A, Culler MD, Turkelson CM, Luciano MG, Thomas CR, Obara N, Groot K, Rivier J, Vale W (1983) In vitro pituitary hormone releasing activity of 40 residue human pancreatic tumor growth hormone releasing factor. Peptides 4: 107.
77. Stachura ME, Tyler JM, Kent PG (1986) Effect of growth hormone-releasing factor-44 upon release of concurrently synthesized hormone by perifused rat pituitary tissue. Endocrinol 119: 1245.
78. Borges JLC, Uskavitch DR, Kaiser DL, Cronin MJ, Evans WS, Thorner MO (1983) Human pancreatic growth hormone-releasing factor-40 (hpGRF-40) allows stimulation of GH release by TRH. Endocrinol 113: 1519.
79. Kato M., Suzuki M (1986) The time course and extracellular Ca^{++} involvement of growth hormone (GH) releasing factor-induced GH secretion in perifused dispersed rat pituitary cells. Jap J Physiol 36: 1225.
80. Adams EF, Winslow CLJ, Mashiter K (1983) Pancreatic growth hormone releasing factor stimulates growth hormone secretion by pituitary cells. Lancet i: 1100.
81. White MC, Daniels M, Kendall-Taylor P, Turner SJ, Mathias D, Teasdale G (1985) Effects of growth hormone-releasing factor (1-44) on growth hormone release from human somatotrophinomas in vitro: interaction with somatostatin, dopamine, vasoactive intestinal peptide and cycloheximide. J Endocrinol 105: 269.
82. Adams EF, Bhuttacharji SC, Halliwell CLJ, Loizou M., Birch G, Mashiter K (1984) Effect of pancreatic growth hormone releasing factors on GH secretion by human somatotrophic pituitary tumors in cell culture. Clin Endocrinol 21: 709.
83. Lamberts SWJ, Verleun T, Oosterom R (1984) The interrelationship between the effects of somatostatin and human pancreatic growth hormone-releasing factor on growth hormone release by cultured pituitary tumor cells from patients with acromegaly. J Clin Endocrinol Metab 58: 250.
84. Labrie F, Gagné B, Lefèvre G (1983) Growth-hormone-releasing factor stimulates adenylate cyclase activity in the anterior pituitary gland. Life Sciences 33: 2229.
85. Schettini G, Cronin MJ, Hewlett EL, Thorner MO, MacLeod RM (1984) Human pancreatic tumor growth hormone-releasing factor stimulates anterior pituitary adenylate cyclase activity, adenosine 3',5'-monoposphate accumulation, and growth hormone release in a calmodulin-dependent manner. Endocrinol 115: 1308.

86. Spada A, Valler L, Giannattasio G (1984) Presence of an adenylate cyclase dually regulated by somatostatin and human pancreatic growth hormone (GH)-releasing factor in GH-secreting cells. Endocrinol 115: 1203.
87. Cronin MJ, Hewlett EL, Evans WS, Thorner MO, Rogol AD (1984) Human pancreatic tumor growth hormone (GH)-releasing factor and cyclic adenosine 3',5'-monophosphate evoke GH release from anterior pituitary cells: the effects of pertussis toxin, cholera toxin, forskolin, and cycloheximide. Endocrinol 114: 904.
88. Culler MD, Kenjo T, Obara N, Arimura A (1984) Stimulation of pituitary cAMP accumulation by human pancreatic GH-releasing factor (1-44). Am J Physiol 247: E609.
89. Login IS, Judd AM, MacLeod RM (1986) Association of $^{45}Ca^{++}$ mobilization with stimulation of growth hormone (GH) release by GH-releasing factor in dispersed normal male rat pituitary cells. Endocrinol 118: 239.
90. Merritt JE, Dobson PRM, Wojcikiewicz RJH, Baird JC, Brown BL (1984) Studies on the involvement of calcium and calmodulin in the action of growth-hormone-releasing factor. Biosc Rep 4: 995.
91. Mougin C, Kehrer P, Gaillard R (1985) Intervention du système calcium-calmoduline dans la libération de l'hormone de croissance provoquée par la somatocrinine. Ann d'Endocrinol Paris 46: 61.
92. Faveur V, Gouin E, Dray F (1985) Growth hormone-releasing factor (GRF) stimulates PGE_2 production in rat anterior pituitary. Evidence for a PGE_2 involvement in GRF-induced GH release. Biochem Biophys Res Comm 126: 725.
93. Cronin MJ, MacLeod RM, Canonico PL (1985) Modification of basal and GRF-stimulated cyclic AMP levels and growth hormone release by phospholipid metabolic enzyme inhibitors. Neuroendocrinol 40: 332.
94. Judd AM, Koike L, MacLeod RM (1985) GRF increases release of growth hormone and arachidonate from anterior pituitary cells. Am J Physiol 248: E438.
95. Cronin MJ, Summers ST, Sortino MA, Hewlett EL (1986) Protein kinase enhances growth hormone releasing factor (1-40) -stimulated cyclic AMP levels in anterior pituitary. J Biol Chem 261: 13932.
96. Escobar DC, Vicentini LM, Ghigo E, Ciccarelli E, Usellini L, Capella C, Cocchi D (1986) Growth hormone-releasing factor does not stimulate phosphoinositides breakdown in primary cultures of rat and human pituitary cells. Acta Endocrinol (Copenh) 112: 345.
97. Frohman LA, Jansson JO (1986) Growth hormone-releasing hormone. Endocr Rev 7: 223.
98. Barinaga M, Yamonoto G, Rivier C, Vale W, Evans R, Rosenfeld MG (1983) Transcriptional regulation of growth hormone gene expresson by growth hormone-releasing factor. Nature 306: 84.
99. Hall K (1971) Effect of intravenous administration of human growth hormone on sulphation factor activity in serum of hypopituitary subjects. Acta Endocrinol (Copenh) 66: 491.
100. Philips LS, Vassilopoulou-Sellin R (1980) Somatomedins. N Engl J Med 302: 371.
101. Philips LS, Vassilopoulou-Sellin R (1980) Somatomedins. N Engl J Med 302: 438.
102. Brazeau P, Guillemin R, Ling N, van Wijk J, Humbel R (1982) Inhibition par les somatomédines de la sécrétion de l'hormone de croissance stimulée par le facteur hypothalamique somatocrinine (GRF) ou le

peptide de synthèse hpGRF. C R Acad Sc Paris. 295 Série III· 651.
103. Ceda GP, Davis RG, Rosenfeld RG, Hoffman AR (1987) The growth hormone (GH) releasing hormone (GHRH)-GH-somatomedin axis· evidence for rapid inhibition of GHRH-elicited GH release by insulin-like growth factors I and II. Endocrinol 120· 1658.
104. Harwood JP, Grewe C, Aguilera G (1984) Actions of growth hormone-releasing factor and somatostatin on adenylate cyclase and growth hormone release in rat anterior pituitary. Mol Cell Endocrinol 37: 277.
105. Law GJ, Ray KP, Wallis M (1985) Effects of growth hormone-releasing factor and somatostatin on growth hormone secretion and cellular cyclic AMP levels. FEBS 179· 12.
106. Aquila MC, McCann SM (1985) Stimulation of somatostatin release in vitro by synthetic human growth hormone-releasing factor by a nondopaminergic mechanism. Endocrinol 117· 762.
107. Aquila MC, McCann SM (1987) Evidence that growth hormone-releasing factor stimulates somatostatin release in vitro via β-endorphin. Endocrinol 120 341.
108. Kraicer J, Cowan JS, Sheppard MS, Lussier B, Moor BC (1986) Effect of somatostatin withdrawal and growth hormone (GH)-releasing factor on GH release in vitro amount available for release after disinhibition. Endocrinol 119 2047.
109. Sheppard MC, Kronheim S, Pimstone BL (1978) Stimulation by growth hormone of somatostatin release from the rat hypothalamus in vitro. Clin Endocrinol 9· 583.
110. Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L (1981) Somatomedin-C mediates growth hormone negative feedback effects on both the hypothalamus and the pituitary. Science 212· 1279.
111. Ono M, Miki N, Miyoshi H (1985) Decreased content and release of hypothalamic growth hormone (GH)- releasing factor (GRF) in rats bearing GH producing GH_3 tumor. 67th Annual Meeting Endocrine Society, Baltimore, Abstr 136.
112. Andhya T, Yokoe T, Brown C, Hutchinson B, Passarelli J, Hollander CS (1986) Immunoreactive growth hormone-releasing factor levels in human and rat peripheral blood. Clin Research 34(2): 713A.
113. Katakami H, Downs TR, Frohman LA (1987) Effect of hypophysectomy on hypothalamic growth hormone-releasing factor content and release in the rat. Endocrinol 120: 1079.
114. Michel D, Lefèvre G, Labrie F (1984) Dexamethasone is a potent stimulator of growth hormone-releasing factor-induced cyclic AMP accumulation in the adenohypophysis. Life Sc 35: 597.
115. Oosterom R, Verleun T, Lamberts SWJ (1984) Human growth hormone-secreting pituitary adenoma cells in long-term culture· effects of dexamethasone and growth hormone releasing factor. J Endocrinol 100: 353.
116. Zeytinoglu FN, Ling N, Mougin C, Esch F (1983) Growth hormone releasing factor stimulates release of neurotensin and efflux of cAMP by a rat C cell line. Endocrinol 112: A293.
117. Zeytin F, Brazeau P (1984) GRF (Somatocrinin) stimulates release of neurotensin, calcitonin and cAMP by a rat C cell line. Biochem Biophys Res Comm 123: 497.
118. Laburthe M, Amiranoff B, Boige Nm, Rouyer-Fessard C, Taremoto K, Moroder L (1983) Interaction of GRF with VIP receptors and stimulation of adenylate cyclase in rat and human intestinal epithelial

membranes. Comparison with PHI and secretin FEBS 159: 89.
119 Pandol SJ, Seifert H, Thomas MW, Rivier J, Vale W (1984) Growth hormone-releasing factor stimulates pancreatic enzyme secretion. Science 225 326
120 Hermansen K, Kappelgaard AM, Esmann J, Ørskov H (1986) Synthetic pancreatic growth hormone-releasing factor (GRF-40) stimulates the secretion of the endocrine pancreas. Diabetes 35: 119.
121. Hermansen K, Kappelgaard AM (1987) Characterization of growth hormone-releasing hormone stimulation of the endocrine pancreas: studies with α- and β-adrenergic and cholinergic antagonists. Acta Endocrinol (Copenh) 114 589.
122. Wehrenberg WB, Ling N, Brazeau P, Esch F, Bohlen P, Baird A, Ying S, Guillemin R (1982) Somatocrinin, growth hormone releasing factor stimulates secretion of growth hormone in anesthetized rats. Biochem Biophys Res Comm 109 382.
123. Wehrenberg WB, Ling N, Bohlen P, Esch F, Brazeau P, Guillemin R (1982) Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. Biochem Biophys Res Comm 109: 562.
124. Wehrenberg WB, Ling N (1983) The absence of an age-related change in the pituitary response to growth hormone-releasing factor in rats. Neuroendocrinol 37 463.
125. Wehrenberg WB, Baird A, Zeytin F, Esch F, Bohlen P, Ling N, Ying SY, Guillemin R (1985) Physiological studies with somatocrinin, a growth hormone-releasing factor. Ann Rev Pharmacol Toxicol 25: 463.
126. Ceda GP, Valenti G, Butturini U, Hoffman AR (1986) Diminished pituitary responsiveness to growth hormone-releasing factor in aging male rats. Endocrinol 118 2109.
127. Sonntag WE, Hylka VW, Meites J (1983) Impaired ability of old male rats to secrete growth hormone in vivo but not in vitro in response to hpGRF (1-44). Endocrinol 113: 2305.
128. Wehrenberg WS, Brazeau P, Luben R, Bohlen P, Guillemin R (1982) Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). Endocrinol 111: 2147.
129. Tannenbaum GS, Epelbaum J, Colle E, Brazeau P, Martin JB (1978) Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion. Endocrinol 102: 1909.
130. Tannenbaum GS, Martin JB (1976) Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinol 98: 562.
131. Wehrenberg WB, Brazeau P, Luben R, Ling N, Guillemin R (1983) A non-invasive functional lesion of the hypothalamo-pituitary axis for the study of growth hormone-releasing factor. Neuroendocrinol 36: 489.
132. Mozi T, Inoue S, Minami S, Egawa M, Wakabayashi J (1986) Episodic growth hormone secretion following exogenous growth hormone-releasing factors in rats. Biomed Res 7: 371.
133. Plotsky P, Vale W (1985) Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophyseal portal circulation of the rat. Science 230. 461.
134. Katakami H, Arimura A, Frohman LA (1986) Growth hormone (GH)-releasing factor stimulates hypothalamic somatostastin release: An inhibitory feedback effect on GH secretion. Endocrinol 118: 1872.
135. Miki N, Ono M (1987) Stimulation of hypothalamic growth hormone-re-

leasing factor release by cysteamine, a potent depletor of somatostatin. 69th Annual Meeting Endocrine Society, Indianapolis, Abstr 118.
136. Wehrenberg WB, Baird A, Ying ST, Ling N (1985) The effects of testosterone and estrogen on the pituitary growth hormone response to growth hormone-releasing factor. Biol Reprod 32: 369.
137. Evans WS, Krieg RJ, Limber ER, Kaiser DL, Thorner MO (1985) Effects of in vivo gonadal hormone environment on in vitro hGRF-40 stimulated GH release. Am J Physiol 249: E276.
138. Ohlsson L, Isaksson O, Jansson JO (1987) Endogenous testosterone enhances growth hormone (GH)-releasing factor-induced GH secretion in vitro. J Endocrinol 113: 249.
139. Ho KY, Leong DA, Sinha YN, Johnson ML, Evans WS, Thorner MO (1986) Sex-related differences in GH secretion in rat using reverse hemolytic plaque assay. Am J Physiol 250: E650.
140. Hoeffler JP, Frawley LS (1986) Capacity of individual somatotropes to release growth hormone varies according to sex: Analysis by reverse hemolytic plaque assay. Endocrinol 119: 1073.
141. Krieg RJ jr, Thorner MO, Evans WS (1986) Sex differences in β-adrenergic stimulaton of growth hormone secretion in vitro. Endocrinol 119· 1339.
142. Janssen JO, Eden S, Isaksson O (1985) Sexual dimorphism in the control of growth hormone secretion. Endocr Rev 6: 128.
143. Shulman DI, Sweetland M, Duckett G, Root AW (1987) Effect of estrogen on the growth hormone (GH) secretory response to GH-releasing factor in the castrate adult female rat in vivo. Endocrinol 120: 1047.
144. Fukata J, Martin JB (1986) Influence of sex steroid hormones on rat growth hormone-releasing factor and somatostatin in dispersed pituitary cells. Endocrinol 119: 2256.
145. Wehrenberg WB, Brazeau P, Ling N, Textor G, Guillemin R (1984) Pituitary growth hormone response in rats during a 24-hour infusion of growth hormone-releasing factor. Endocrinol 114: 1613.
146 Wehrenberg WB (1986) Continuous infusion of growth hormone-releasing factor: effects on pulsatile growth hormone secretion in normal rats. Neuroendocrinol 43: 391.
147 Jansson J, Carlsson L, Isaksson OGP (1985) Growth hormone (GH)-releasing factor (GRF) pretreatment enhances the GRF-induced GH secretion in rats with the pituitary autotransplanted to the kidney capsule. Endocrinol 116: 95.
148. Clark RG, Robinson CAF (1985) Growth induced by pulsatile infusion of an amidated fragment of human growth hormone releasing factor in normal and GHRH-deficients rats. Nature 314: 281.
149. Bilezikjian LM, Vale WW (1984) Chronic exposure of cultured rat anterior pituitary cells to GRF causes partial loss of responsiveness to GRF. Endocrinol 115: 2032.
150. Seiffert H, Bilezikjian LM, Rivier J, Vale W (1985) GRF regulates the number of its binding sites in rat anterior pituitary cells. 67th Annual Meeting Endocrine Society, Baltimore, Abstr 140.
151. Diequez C, Foord SM, Shewring G, Edwards CA, Heyburn PJ, Peters JR, Hall R, Scanlon MF (1984) The effects of long term growth hormone releasing factor (GRF 1-40) administration on growth hormone secretion and synthesis in vivo. Biochem Biophys Res Commun 121: 111.
152. Simard J, Labrie F (1986) Characteristics of desensitization of growth hormone and cyclic AMP responses to growth hormone-releasing factor and prostaglandin E_2 in rat anterior pituitary cells in

culture. Mol Cell Endocrinol 46: 79.
153. Ceda GP, Hoffman AR (1985) Growth hormone-releasing factor desensitization in rat anterior pituitary cells in vitro. Endocrinol 116: 1334.
154. Clayton RN, Bailey LC (1987) Somatostatin partially reverses desensitization of somatotrophs induced by growth hormone-releasing factor. J Endocrinol 112: 69.
155. Eden S, Eriksson E, Martin JB, Modigh K (1981) Evidence for a growth hormone releasing factor mediating alpha-adrenergic influence on growth hormone secretion in the rat. Neuroendocrinol 33: 24.
156. Katakami H, Kato Y, Matsushita N, Imura H (1984) Effects of neonatal treatment with monosodium glutamate on growth hormone release induced by clonidine and prostaglandin E_1 in conscious male rats. Neuroendocrinol 38: 1.
157. Miki N, Ono M, Shizume K (1984) Evidence that opiatergic and α-adrenergic mechanisms stimulate rat growth hormone release via growth hormone-releasing factor (GRF). Endocrinol 114: 1950.
158. Cella SG, Locatelli V, De Gennaro V, Pellini C, Pintor C, Muller EE (1986) In vivo studies with growth hormone (GH)-releasing factor and clonidine in rat pups: Ontogenetic development of their effect on GH release and synthesis. Endocrinol 119: 1164.
159. Locatelli V, Torsello A, Redaelli M, Chigo E, Massara F, Müller EE (1986) Cholinergic agonist and antagonist drugs modulate the growth hormone response to growth hormone-releasing hormone in the rat: evidence for mediation by somatostatin. J Endocrinol 111: 271.
160. Kakucska I, Makara GB (1983) Various putative neurotransmitters affect growth hormone (GH) release in rats with anterolateral hypothalamic deafferentiation of the medial basal hypothalamus: Evidence for mediation by a GH-releasing factor. Endocrinol 113: 318.
161. Murakami Y, Kato Y, Kabayama J, Tojo K, Inoue T, Imura H (1985) Involvement of growth hormone-releasing factor in growth hormone secretion induced by gamma-aminobutyric acid in conscious rats. Endocrinol 117: 787.
162. Fiók J. Acs Z, Makara G, Erdö SL (1984) Site of γ-aminobutyric acid (GABA)-mediated inhibition of growth hormone secretion in the rat. Neuroendocrinol 39: 510.
163. Murakami Y, Kato Y, Kabayama Y, Inoue T, Tojo K, Ortha H, Imura H (1985) Inhibition by antiserum to rat growth hormone-releasing factor of growth hormone secretion induced by a met-enkephalin analog, FK 33-824, in rats. Proc Soc Exp Biol Med 178: 1512.
164. Wehrenberg WB, Bloch B, Ling N (1985) Pituitary secretion of growth hormone in response to opioid peptides and opiates is mediated through growth hormone-releasing factor. Neuroendocrinol 41: 13.
165. Murakami Y, Kato Y, Kabayama Y, Tojo K, Inoue T, Imura H (1986) Involvement of growth hormone (GH)-releasing factor in GH secretion induced by serotoninergic mechanisms in conscious rats. Endocrinol 119: 1089.
166. Imaki T, Shibasaki T, Masuda A, Hotta M, Yamanchi N, Demura H, Shizume K, Wakabayashi I, Ling N (1986) The effect of glucose and free fatty acids on growth hormone (GH)-releasing factor-mediated GH secretion in rats. Endocrinol 118: 2390.
167. Locatelli V, Miyoshi H, Bestetti G, Rossi GL, Müller EE (1985) Effect of growth hormone-releasing stimuli in streptozotocin diabetic rats. Brain Res 341: 35.

168. Katakami H, Downs TR, Frohman LA (1986) Decreased hypothalamic growth hormone-releasing hormone content and pituitary responsiveness in hypothyroidism. J Clin Invest 77: 1704.
169. Root AD, Shulman D, Root J, Diamond F (1986) The interrelationships of thyroid and growth hormones: effect of growth hormone releasing hormone in hypo- and hyperthyroid male rats. Acta Endocrinol (Copenh) Suppl 279: 367.
170. Tannenbaum GS (1987) Role of glucocorticoids in regulation of pulsatile growth hormone secretion. 69th Annual Meeting Endocrine Society, Indianapolis, Abstr 921.
171. Attanasio A, Bombelli M, Kuzmanovic D, Gupta D (1986) The pineal influence on growth hormone secretion. Neuroendocrinol Lett 8: 275.
172. Lengyel AJ, Tannenbaum GS (1987) Mechanisms of calcitonin-induced growth hormone (GH) suppression: roles of somatostatin and GH-releasing factor. Endocrinol 120 1377.
173. Vaccarino FJ, Bloom FE, Rivier J, Vale W, Koob GF (1985) Stimulation of food intake in rats by centrally administered hypothalamic growth hormone-releasing factor. Nature 314: 167.
174. Imaki T, Shibasaki T, Hotta M, Masuda A, Demura H, Schizume K, Ling N (1985) The satiety effect of growth hormone-releasing factors in rats. Brain Res 340: 186.
175. Ehlers CL, Reed TK, Hendriksen SJ 1986) Effects of corticotropin-releasing factor and growth hormone-releasing factor on sleep and activity in rats. Neuroendocrinol 42: 467.
176. Hammer RE, Brinster RL, Rosenfeld MG, Evans RM, Mayo KE (1985) Expression of human growth hormone-releasing factor in transgenic mice results in increased somatic growth. Nature 315: 413.
177. Accary JP, Dubrasquet M, Lehy T, Lewin MJM (1986) Growth hormone releasing hormone stimulates the release of gastrin in rat. Peptides 7: 241.
178. Goffette D, de la Tour J, Chariot J, Roze C (1986) Effect of rGRF on exocrine pancreatic secretion of the rat in vivo. Peptides 7: 245.
179. Dubreuil P, Morisset J (1986) Effect of somatocrinin and a somatostatin antiserum on duodenal and gastric growth in the rat. Reg Peptides 16: 51.
180. Thorner MO, Rivier J, Spiess J, Borges JL, Vance ML, Bloom SR, Rogol AD, Cronin MJ, Kaiser DL, Evans WS, Webster JD, MacLeod RM, Vale W (1983) Human pancreatic growth-hormone-releasing factor selectively stimulates growth-hormone secretion in man. Lancet 1: 25.
181. Vance ML, Borges JLC, Kaiser DL, Evans WS, Furlanetto R, Thominet JL, Frohman LA, Rogol AD, MacLeod RM, Bloom S, Rivier J, Vale W, Thorner MO (1984) Human pancreatic tumor growth hormone-releasing factor Dose-response relationships in normal man. J Clin Endocrinol Metab 58: 838.
182. Gelato MC, Pescovitz OH, Cassorla F, Loriaux DL, Merian GR (1984) Dose-response relationships for the effects of growth hormone-releasing factor (1-44)-NH_2 in young adult men and women. J Clin Endocrinol Metab 59: 197.
183. Losa M, Stalla GK, Muller OA, van Werder K (1983) Human pancreatic growth hormone releasing factor (hpGRF): Dose-response of GRF- and GH levels. Klin Wschr 61: 1249.
184. Sassolas G, Biot-Laporte S, Cohen R, Chatelain P, Boissel JP, Ferry S, Chayvialle JA, Dupin P, Garry P, Claustrat B, Girard P, Borson F, Trouillas J, Loras B (1986) Clinical studies with human growth

hormone releasing factor in normal adults and patients. Peptides 7, suppl. 1: 281.
185. Evans WS, Borges JLC, Vance ML, Kaiser DL, Rogol AD, Furlanetto R, Rivier J, Vale W, Thorner MO (1984) Effects of human pancreatic growth hormone releasing factor-40 on serum growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone and somatomedin-C concentrations in normal women throughout the menstrual cycle. J Clin Endocrinol Metab 59: 1006.
186. Egli CA, Hauffa BP, Kaplan SL, Grumbach M (1984) Pituitary growth hormone (GH) response to growth hormone releasing-factor (GHRF-44) differs during the menstrual cycle. 7th Int Congr Endocrinol, Quebec, p 603, Abstr 685.
187. Frohman LA, Thominet JL, Webb CB, Vance ML, Uderman H, Rivier J, Vale W, Thorner MO (1984) Metabolic clearance and plasma disappearance rates of human pancreatic tumor growth hormone releasing factor in man. J Clin Invest 73: 1304.
188. Leppalkoto J, Virkunen P, Lybeck H (1972) Elimination of TRH in man. J Clin Endocrinol Metab 35: 477.
189. Redding TW, Kastin AJ, Gonzalez-Barcena D, Coy DH, Coy EJ, Schalch DS, Schally AV (1973) The half-life, metabolism and excretion of titriated luteinizing hormone-releasing hormone (LH-RH) in man. J Clin Endocrinol Metab 37: 626.
190. Shepparo M, Shapiro B, Pimstone B, Kronheim S, Berelowitz M, Gregory M (1979) Metabolic clearance and plasma half-disappearance time of exogenous somatostatin in man. J Clin Endocrinol Metab 48: 50.
191. Frohman LA, Downs TR, Williams TC, Heimer EP, Pan YE, Felix AM (1986) Rapid enzymatic degradation of growth hormone-releasing hormone by plasma in vitro and in vivo to a biologically inactive product cleaved at the NH_2 terminus. J Clin Invest 78: 906.
192. Shibasaki T, Shizume K, Nakahara M, Masuda A, Jigiki K, Demura H, Wakabayashi I, Ling N (1984) Age-related changes in plasma growth hormone response to growth hormone-releasing factor in man. J Clin Endocrinol Metab 58: 212.
193. Pavlow EP, Harman SM, Merriam GR, Gelato MC, Blackman MR (1986) Responses of growth hormone (GH) and somatomedin-C to GH-releasing hormone in healthy aging men. J Clin Encdocrinol Metab 62: 595.
194. Schirock EA, Lustig RH, Rosenthal SM, Kaplan SL, Grumbach MM (1984) Effect of growth hormone (GH)-releasing hormone (GRH) on plasma GH in relation to the magnitude and duration of GH deficency in 26 children and adults with isolated GH deficiency or multiple pituitary hormone deficiencies: Evidence for hypothalamic GRH deficiency. J Clin Endocrinol Metab 58: 1043.
195. van Vliet G, Bosson D, Robijn C, Craen M, Malvaux P, Vanderschueren-Lodewijks M, Wolter R (1985) Effect of growth hormone-releasing factor on plasma growth hormone, prolactin and somatomedin C in hypopituitary and short normal childrens. Hormone Res 22: 32.
196. Penny ES, Penman E, Price J, Rees LH, Sopwith AM, Wass JAH, Lytras N, Besser GM (1984) Circulating growth hormone releasing factor concentrations in normal subjects and patients with acromegaly. Br Med J 289: 453.
197. Sopwith AM, Penny ES, Besser GM, Rees LH (1985) Stimulation by food of peripheral plasma immunoreactive growth hormone releasing factor. Clin Endocrinol 22: 337.
198. Sopwith AM, Penny ES, Lytras N, Besser GM, Rees LH (1987) Dissocia-

tion between circulating concentrations of immunoreactive growth hormone releasing factor and growth hormone in normal human subjects. Clin Science 72: 181.
199. Knip M, Tapanainen P, Rautala H, Leppaluoto J, Lautalla P, Kaar ML (1987) Sleep-induced release of growth hormone and growth hormone releasing hormone in normal children. 69th Annual Meeting Endocrine Society, Indianapolis, Abstr 111.
200. Chihara K, Kashio Y, Kita T, Okimura Y, Kaji H, Abe H, Fujita T (1986) L-Dopa stimulates release of hypothalamic growth hormone-releasing hormone in humans. J Clin Endocrinol Metab 62: 466.
201. Chihara K, Kashio Y, Abe H, Minamitani N, Kaji H, Kita T, Fujita T (1985) Idiopathic growth hormone GH) deficiency, and GH deficiency secondary to hypothalamic germinoma: Effect of single and repeated administration of human GH-releasing factor (hGRF) on plasma GH levels and endogenous hGRF-like immunoreactivity level in cerebrospinal fluid. J Clin Endocrinol Metab 60: 269.
202. Penny ES, Sopwith AM, Patience RL, Wass JAH, Besser GM, Rees LH (1986) Characterization by high-performance liquid chromatography of circulating growth hormone-releasing factors in normal subjects. J Endocrinol 111: 507.
203. Davies RR, Turner S, Johnston DG (1984) Oral glucose inhibits growth hormone secretion induced by human pancreatic growth hormone releasing factor 1-44, in normal man. Clin Endocrinol 21: 477.
204. Sharp PS, Foley K, Chahal P, Kohner EM (1984) The effect of plasma glucose on the growth hormone response to human pancreatic growth hormone releasing factor in normal subjects. Clin Endocrinol 20: 497.
205. Masuda A, Shibasaki T, Nakahara M, Imaki T, Kiyosawa Y, Jibiki K, Demura H, Shizume K, Ling N (1985) The effect of glucose on growth hormone (GH)-releasing hormone-mediated GH secretion in man. J Clin Endocrinol Metab 60: 523.
206. Imaki T, Shibasaki T, Shizume K, Masuda A, Hotta M, Kiyosawa Y, Jigiki K, Demura H, Tsushima T, Ling N (1985) The effect of free fatty acids on growth hormone (GH)-releasing hormone-mediated GH secretion in man. J Clin Endocrinol Metab 60: 290.
207. Sartorio A, Spada A, Bochiccio D, Atterrato A, Morabito F, Faglia G (1986) Effect of thyrotropin-releasing hormone on growth hormone release in normal subjects pretreated with human pancreatic growth hormone-releasing factor 1-44 pulsatile administration. Neuroendocrinol 44: 470.
208. Sheldon WR jr, DeBold CR, Evans WS, Decherney GS, Jackson RV, Island DP, Thorner MO, Orth DN (1985) Rapid sequential intravenous administration of four hypothalamic releasing hormones as a combined anterior pituitary function test in normal subjects. J Clin Endocrinol Metab 60: 623.
209. Looy BJ, Nieuwenhuijzen Kruseman AC, Mudde AH, Fröhlich M, Piaditis GP, Hodgkinson SC, McLean C, Grossman A, Coy DH, Rees LH, Besser GM (1986) The interaction of growth hormone releasing hormone with other hypothalamic hormones on the release of anterior pituitary hormones. Clin Endocrinol 24: 149.
210. Cohen R, Bouquier D, Biot-Laporte S, Vermeulen E, Claustrat B, Cherpin MH, Cabrera P, Guidetti P, Ferry S, Bizollon CH A, Sassolas G (1986) Pituitarty stimulation by combined administration of four hypothalamic releasing hormones in normal men and patients. J Clin Endocrinol Metab 62: 892.

211. Massara F, Chico E, Goffis S, Molinatti GM, Müller EE, Camanni F (1984) Blockade of hp-GRF-40-induced GH release in normal men by a cholinergic muscarinic antagonist. J Clin Endocrinol Metab 59: 1025.
212. Casanueva FF, Villaenueva L, Diaz Y, Devesa J, Fernandex-Cruz A, Schally AV (1986) Atropine selectively blocks GHRH-induced GH secretion without altering LH, FSH, TSH, PRL and ACTH/cortisol secretion elicited by their specific hypothalamic releasing factors. Clin Endocrinol 25: 319.
213. Jordan V, Dieguez C, Lafaffiau I, Rodriguez-Arnao MD, Gomez-Pan A, Hall R, Scanlon MF (1986) Influence of dopaminergic adrenergic and cholinergic blockade and TRH administration on GH responses to GRF 1-29. Clin Endocrinol 24: 291.
214. Yagi H, Nagashima T, Noji T, Junoki H, Susuki A, Kuroume T (1986) Inhibitory effect of antimuscarinic cholinergic drug (atropine) on growth hormone (GH) secretion induced by GH-releasing factor. Horm Metab Res 18: 723.
215. Vance ML, Kaiser DL, Frohman LA, Rivier J, Vale W, Thorner MO (1987) Role of dopamine in the regulation of growth hormone secretion: dopamine and bromocriptine augment growth hormone (GH)-releasing hormone stimulated GH secretion in normal man. J Clin Endocrinol Metab 64: 1136.
216. Giusti M, Mazzochi G, Sessarego P, Mignone D, Lomeo A, Monachesi M, Giordano G (1984) Effects of GRF(1-40) and dromperidone on GH secretion in normal man. Clin Endocrinol 21: 339.
217. Chihara K, Kodama H, Kaji H, Kita T, Kashio Y, Okimura Y, Abe H, Fujita T (1985) Augmentation by propranolol of growth hormone-releasing hormone-(1-44)-NH_2-induced growth hormone release in normal short and normal children. J Clin Endocrinol Metab 61: 229.
218. Valcavi R, Dieguez C, Azzarito C, Edwards CA, Dotti C, Page MD, Portioli I, Scanlon MF (1987) Effect of oral administration of melatonin on GH responses to GRF 1-44 in normal subjects. Clin Endocrinol 26: 453.
219. Rosenthal SM, Hulse JA, Kaplan SL, Grumbach MM (1986) Exogenous growth hormone inhibits growth hormone-releasing factor-induced growth hormone secretion in normal men. J Clin Invest 77: 170.
220. Ross RJM, Borges F, Grossman A, Smith R, Ngahfoong L, Rees LH, Savage MO, Besser GM (1987) Growth hormone pretreatment in man blocks the response to growth hormone-releasing hormone: evidence for a direct effect of growth hormone. Clin Endocrinol 26: 117.
221. Hanew K, Goh M, Sato S, Shimizu Y, Yoshinaga K (1987) Acute and chronic administration of growth hormone (GH) inhibits GH-releasing hormone-44 (GRH)-induced GH secretion in patients with idiopathic GH deficiency. 69th Annual Meeting Endocrine Society, Indianapolis, Abstr 786.
222. Gelato MC, Rittmaster RS, Pescovitz OH, Nicoletti MC, Nixon WE, D'Agath R, Loriaux DL, Merrian GR (1985) Growth hormone responses of continuous infusions of growth hormone-releasing hormone. J Clin Endocrinol Metab 61: 223.
223. Vance ML, Kaiser DL, Evans WS, Thorner MO, Furlanetto RT, Rivier J, Vale W, Perisutti G, Frohman LA (1985) Evidence for a limited growth hormone (GH)-releasing hormone (GHRH)-releasable quantity of GH: Effects of 6-hour infusions of GHRH on GH secretion in normal man. J Clin Endocrnol Metab 60: 370.
224. Vance ML, Kaiser DL, Rivier J, Vale W, Thorner MO (1986) Dual effects

of growth hormone (GH)-releasing hormone infusion in normal men: somatotroph desensitization and increase in releasable GH. J Clin Endocrinol Metab 62: 591.
225. Schulte HM, Allolio B, Frohwein US, Beuker G, Reinwein D (1986) Continuous infusion of growth hormone releasing hormone does not alter the growth hormone response to different stress stimuli. Clin Endocrinol 25: 511.
226. Losa M, Bock L, Schopohl J, Stalla GK, Müller OA, von Werder K (1984) Growth hormone releasing factor infusion does not sustain elevated GH-levels in normal subjects. Acta Endocrinol (Copenh) 107: 462.
227. Massara F, Chigo E, Molinatti P, Mazza E, Locatelli V, Müller EE, Camanni F (1986) Potentiation of cholinergic tone by pyridostigmine bromide re-instates and potentiates the growth hormone responsiveness to intermittent administration of growth hormone-releasing factor in man. Acta Endocrinol (Copenh) 113: 12.
228. Borges JLC, Blizzaro RM, Gelato MC, Furlanetto R, Rogol AD, Evans WS, Vance ML, Kaiser DL, MacLeod RM, Merriam GR, Loriaux DL, Spiess J, Rivier J, Vale W, Thorner MO (1983) Effects of human pancreatic tumor growth hormone releasing factor on growth hormone and somatomedin C levels in patients with idiopathic growth hormone deficiency. Lancet ii: 119.
229. Pintor C, Loche S, Puggioni R, Cella SG, Locatelli V, Villa F, Corda R, Müller EE (1986) Growth hormone response to hpGRF-40 in different forms of growth retardation and endocrine metabolic diseases. Eur J Pediatr 144: 475.
230. Gelato MC, Malozowski S, Caruso-Nicoletti M, Ross JL, Pescovitz OH, Rose S, Loriaux DL, Cassorla F, Merriam GR (1986) Growth hormone (GH) responses to GH-releasing hormone during pubertal development in normal boys and girls: Comparison to idiopathic short stature and GH deficiency. J Clin Endocrinol Metab 63: 174.
231. Takano K, Hizuka N, Shizume K, Ling NC (1984) Plasma GH response to hpGRF-44 in normal children of short stature and patients with GH deficiency. Endocrinol Japon 31: 41.
232. Takamo K, Hizuka N, Shizume K, Asakawa K, Miyakawa M, Hiroxse N, Shibasaki T, Linfg N (1984) Plasma growth hormone (GHJ) response to GH-releasing factor in normal children with short stature and patients with pituitary dwarfism. J Clin Endocrinol Metab 58: 236.
233. Pertzelan A, Keret R, Bauman B, Ben-Zeev Z, Olsen DB, Szoke B, Comaru-Schally AM, Schally AV, Laron Z (1985) Plasma growth hormone response to synthetic GH-RH_{1-44} in 52 children and adults with growth hormone deficiency of various etiologies. Hormone Res 22: 24.
234. Pintor C, Fanni V, Loche S, Locatelli V, Cella SG, Villa F, Minuto F, Corda R, Muller EE (1983) Synthetic $hpGRF_{1-40}$ stimulates growth hormone and inhibits prolactin secretion in normal children and children with isolated growth hormone deficiency. Peptides 4: 929.
235. Butenandt O, Emmlinger M, Doerr H (1986) Single and repeated testing of growth hormone secretory capacity in hypopituitarism using growth hormone releasing factor. Acta Endocrinol (Copenh) Suppl 279: 118.
236. Takano K, Hizuka N, Shizume K, Honda N, Ling NC (1985) Plasma growth hormone (GH) responses to single and repetitive subcutaneous administration of GH releasing factor (hpGRF-44) in normal and GH deficient children. Acta Endocrinol (Copenh) 108: 11.
237. Borges JLC, Blizzaro RM, Evans WS, Furlanetto R, Rogol AD, Kaiser DL, Rivier J, Vale W, Thorner MO (1984) Stimulation of growth hormone

(GH) and somatomedin C in idiopathic GH-deficient subjects by intermittent pulsatile administration of synthetic human pancreatic tumor GH-releasing factor. J Clin Endocrinol Metab 59: 1.
238. Thorner MO, Reschke J, Chitwood J, Rogol AD, Furlanetto R, Rivier J, Vale W, Blizzard RM (1985) Acceleration of growth in two children treated with human growth hormone-releasing factor. N Engl J Med 312: 4.
239. Valk JM, Langhout-Chabloz ANE, Smals AGH, Kloppenborg PWC (1983) Accurate measurement of the lower leg length and the ulnar length and its application in short term growth measurements. Growth 47: 53.
240. Gelato MC, Ross JL, Malozowski S, Pescovitz OH, Skerda M, Cassorla F, Loriaux DL, Merriam GH (1985) Effects of pulsatile administration of growth hormone (GH)-releasing hormone on short term linear growth in children with GH deficiency. J Clin Endocrinol Metab 61: 444.
241. Smith PJ, Brook CGD, Rivier J, Vale W, Thorner MO (1986) Nocturnal pulsatile growth hormone releasing hormone treatment in growth hormone deficiency. Clin Endocrinol 25: 35.
242. Ross RJN, Rodda C, Tsagarakis S, Davies PSW, Grossman A, Rees LH, Preece MA, Savage MO (1987) Treatment of growth-hormone deficiency with growth-hormone-releasing hormone. Lancet i: 5.
243. Bercu BB, Shulman D, Root AW, Spiliotis BE (1986) Growth hormone (GH) provocative testing frequently does not reflect endogenous GH secretion. J Clin Endocrinol Metab 63: 709.
244. Spiliotis BE, August GP, Hung W, Sonis W, Mendelson W, Bercu BB (1984) Growth hormone neurosecretory dysfunction. JAMA 251: 2223.
245. Loche S, Corda R, Lampis A, Puggioni R, Cella SG, Muller EE, Pintor C (1986) The effect of oxandrolone on the growth hormone response to growth hormone releasing hormone in children with constitutional growth delay. Clin Endocrinol 25: 195.
246. Press M, Tamborlane WV, Thorner MO, Vale W, Rivier J, Gertner JM (1984) Pituitary response to growth hormone-releasing factor in diabetes. Failure of glucose-mediated suppression. Diabetes 33: 804.
247. Sharp PS, Foley K, Kohner EM (1984) Evidence for a central abnormality in the regulation of growth hormone secretion in insulin-dependent diabetes. Diab Med 1: 205.
248. Kaneko K, Komine S, Maeda T, Ohta M, Tsuchima T, Shizume K (1985) Growth hormone responses to growth-hormone-releasing hormone and thyrotropin-releasing hormone in diabetic patients with and without retinopathy. Diabetes 34: 710.
249. Williams T, Berelowitz M, Joffe SN, Thorner MO, Rivier J, Vale W, Frohman LA (1984) Impaired growth hormone responses to growth hormone-releasing factor in obesity. A pituitary defect reversed with weight reduction. N Engl J Med 311: 1403.
250. Kopelman PG, Noonan K, Goulton R, Forrest J (1985) Impaired growth hormone response to growth hormone releasing factor and insulin-hypoglycemia in obesity. Clin Endocrnol 23: 87.
251. Kopelman PG, Noonan K (1986) Growth hormone response to low dose intravenous injections of growth hormone releasing factor in obese and normal weight women. Clin Endocrinol 24: 157.
252. van Vliet G, Bosson D, Rummens E, Robijn C, Wolter R (1986) Evidence against growth hormone-releasing factor deficiency in children with idiopathic obesity. Acta Endocrinol (Copenh) Suppl 279: 403.
253. Rosskamp R, Becker M, Soetadji S (1987) Circulating somatomedin-C levels and the effect of growth hormone-releasing factor on plasma

levels of growth hormone and somatostatin-like immunoreactivity in obese children. Eur J Pediatr 146: 48.
254. Williams T, Maxon H, Thorner MO, Frohman LH (1985) Blunted growth hormone (GH) response to GH-releasing hormone in hypothyroidism resolves in the euthyroid state. J Clin Endocrinol Metab 61: 454.
255. Valcavi R, Jordan V, Dieguez C, John R, Manicardi E, Partioli I, Rodriguez-Arnao MD, Gomex-Pan A, Hall R, Scanlon MF (1986) Growth-hormone responses to GRF 1-29 in patients with primary hypothyroidism before and during replacement therapy with thyroxine. Clin Endocrinol 24: 693.
256. Melmed S, Braunstein GF, Horvath E, Ezrin C, Kovacs K (1983) Pathophysiology of acromegaly. Endocr Rev 4: 271.
257. Mazie P (1886) On two cases of acromegaly: marked hypertrophy of the upper and lower limbs and the head. Revue Med (Paris) 6: 297.
258. Minkowski O (1987) Uber einen Fall von Akromegalie. Berl Klin Wochenschr 24: 371.
259. Cushing HC (1909) Partial hypophysectomy for acromegaly. Ann Surgery 50: 1002.
260. Evans HM, Long JA (1921) The effect of the anterior lobe of the pituitary administered intraperitoneally upon growth, maturity and oestrus cycle of the rat. Anat Rec 21: 62.
261. Li CH, Papkoff H (1956) Preparation and properties of growth hormone from human and monkey pituitary glands. Science 124: 1293.
262. Burrow GN, Wortzman G, Rewcastle NB et al.(1981) Microadenomas of the pituitary and abnormal sellar tomograms in an unselected autopsy series. N Engl J Med 304: 156.
263. Melmed S (1986) Pituitary tumors secreting growth hormone and prolactin. Ann Int Med 105: 238.
264. Faglia G, Arosio M, Ambrosi B (1985) Recent advances in diagnosis and treatment of acromegaly, in Imura H (ed): The pituitary gland. New York, Raven Press, page 363-403.
265. Pieters GFFM, Romeijn JE, Smals AGH, Kloppenborg PWC (1982) Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. J Clin Endocrinol Metab 54: 942.
266. Hanew K, Kokubun M, Sasaki A, Mouri T, Yoshinaga K (1980) The spectrum of pituitary growth hormone responses to pharmacological stimuli in acromegaly. J Clin Endocrinol Metab 51: 292.
267. Kelijman M, Williams TC, Downs TR, Frohman LA (1987) Comparison of the sensitivity of growth hormone (GH) secretion to somatostatin (SRIF) in vivo and in vitro in acromegaly. Clin Research 35: 585A.
268. Arafah BUM, Bradkey JS, Kaufman B, Velasco M, Manni A, Pearson OH (1980) Transsphenoidal microsurgery in the treatment of acromegaly and gigantism. J Clin Endocrinol Metab 50: 578.
269. Feingold KR, Goldfine ID, Weinstein PR (1979) Acromegaly with normal growth hormone levels and pituitary histology. Case report. J Neurosurg 50: 503.
270. Webb CB, Thominet JL, Frohman LA (1983) Ectopic growth hormone releasing factor stimulates growth hormone release from human somatotroph adenomas in vitro. J Clin Endocrinol Metab 56: 417.
271. Cryer PE, Daughaday WH (1969) Regulation of growth hormone secretion in acromegaly. J Clin Endocrinol Metab 29: 386.
272. Lawrence AM, Goldfine ID, Kirsteins L (1970) Growth hormone dynamics in acromegaly. J Clin Endocrinol Metab 31: 239.
273. Liuzzi A, Chiodini PG, Botalla L, Cremascoli G, Silvestrini F (1972)

Inhibitory effect of L-Dopa on GH-release in acromegalic patients. J Clin Endocrinol Metab 35: 941.
274. Boyd AE, Lebovitz HE, Pfeiffer JB (1970) Stimulation of human growth hormone secretion by L-Dopa. N Engl J Med 283: 1425.
275. Smythe GA, Compton PJ, Lazarus L (1976) Serotoninergic control of human growth hormone secretion: the actions of L-Dopa and 2-bromo-alfa-ergocryptine. In Pecile A, Muller EE (eds): Growth hormone and related peptides. Excerpta Medica, Amsterdam, page 222-235.
276. Marcovitz S, Goodyer CG, Guyda H, Gardiner RJ, Hardy J (1982) Comparative study of human fetal, normal adult and somatotropic adenoma pituitary function in tissue culture. J Clin Endocr Metab 54: 6.
277. Mashiter K, van Noorden S, De Marco L, Adams E, Joplin GF (1979) Hormone secretion by human somatotrophic, lactotrophic, and mixed pituitary adenomas in culture. J Clin Endocrinol Metab 48: 108.
278. Dasmahapatra A, Urdanavia E, Cohen MP (1981) Growth hormone response to thyrotropin-releasing hormone in diabetes. J Clin Endocrinol Metab 52: 859.
279. Maeda K, Kato Y, Ohgo S, Chihara K, Yoshimoto Y, Yamaguchi N, Kuromaru S, Imura H (1976) Growth hormone release following thyrotropin releasing hormone injection into patients with anorexia nervosa. Acta Endocrinol (Copenh) 81: 1.
280. Gonzalez-Barcena D, Kastin AJ, Schalch DS, Torres-Zamora M, Perez-Pasten E, Kato A, Schally A (1973) Responses to thyrotropin-releasing hormone in patients with renal failure and after infusion in normal men. J Clin Endocrinol Metab 36: 117.
281. Panerai AE, Salerno F, Manneschi M, Cocchi D, Muller EE (1977) Growth hormone and prolactin response to thyrotropin-releasing hormone in patients with severe liver disease. J Clin Endocrinol Metab 45: 134.
282. Collu R, Leboeuf G, Letarte J, Ducharme JR (1977) Increase in plasma growth hormone levels following thyrotropin-releasing hormone injection in children with primary hypothyroidism. J Clin Endocrinol Metab 44: 743.
283. Maeda K, Kato Y, Ohgo S, Chihara K, Yoshimoto Y, Yamaguchi N, Kuromaru S, Imura H (1975). Growth hormone and prolactin release after injection of thyrotropin releasing hormone in patients with depression. J Clin Endocrinol Metab 40: 501.
284. Wood SM, Ch'ng JLC, Adams EF, Webster JD, Joplin GF, Mashiter K, Bloom SR (1983) Abnormalities of growth hormone release in response to human pancreatic growth hormone releasing factor (GRF_{1-44}) in acromegaly and hypopituitarism. Br Med J 286: 1687.
285. Gelato MC, Merriam GR, Vance ML, Goldman JA, Webb C, Evans WS, Rock J, Oldfield EH, Molitch ME, Rivier J, Vale W, Reichlin S, Frohman LA, Loriaux DL, Thorner MO (1985) Effects of growth hormone-releasing factor on growth hormone secretion in acromegaly. J Clin Endocrinol Metab 60: 251.
286. Losa M, Schopohl J, Stalla GK, Müller OA, von Werder K (1985) Growth hormone releasing factor-test in acromegaly: comparison with other dynamic tests. Clin Endocrinol 23: 99.
287. Losa M, Stalla GK, Müller OA, Hartt R, von Werder K (1984) GH-stimulation with growth hormone releasing factor ($hpGRF_{1-44}$) in acromegaly before and after transsfenoidal operation. 7th Int.Congress of Endocrinol, Ouebec, page 981, Abstr 1441.
288. Shibasaki T, Shizume K, Masuda A, Nakahara M, Hizuka N, Miyakawa M, Takano K, Demura H, Wakabayashi I, Ling N (1984) Plasma growth

hormone response to growth hormone-releasing factor in acromegalic patients. J Clin Endocrinol Metab 58: 215.
289. Chiodini PG, Liuzzi A, Dallabonzana D, Opizzi G, Verde GP (1985) Changes in growth hormone (GH) secretion induced by human pancreatic GH-releasing hormone-44 in acromegaly: A comparison with thyrotropin-releasing hormone and bromocriptine. J Clin Endocrinol Metab 60: 48.
290. Schulte HN, Beuker G, Windeck R, Olbright T, Reinwein D (1985) Failure to respond to growth hormone releasing hormone (GHRH) in acromegaly due to a GHRH secreting pancreatic tumor: dynamics of multiple endocrine testing. J Clin Endocrinol Metab 61: 585.
291. Barkan AL, Shenker Y, Grekin RJ, Vale W, Lloyd RV, Beals TF (1986) Acromegaly due to ectopic growth hormone (GH)-releasing hormone (GHRH) production. Dynamic studies of GH and ectopic GHRH secretion. J Clin Endocrinol Metab 63: 1057.
292. Thorner MO, Frohman LA, Leong DA, Thominet J, Downs T, Hellmann P, Chitwood J, Vaughan JM, Vale W et al (1984) Extrahypothalamic growth-hormone-releasing factor (GRF) secretion is a rare cause of acromegaly: Plasma GRF levels in 177 acromegalic patients. J Clin Endocrinol Metab 59: 846.
293. Arosio M, Ambrosi B, Guglielmino L, Faglia G (1985) Human pancreatic growth-hormone releasing factor (hpGRF-44) in acromegaly before and after adenomectomy. Modifications induced by somatostatin (GHRIH) infusion. J Endocrinol Invest 8: 449.
294. Losa M, Schopohl J, Muller OA, von Werder K (1985) Growth hormone releasing factor induces prolactin secretion in acromegalic patients but not in normal subjects. Acta Endocrinol (Copenh) 109: 467.
295. Cozzi R, Dallabonzana D, Opizzi G, Verde G, Liuzzi A, Chiodini PG (1986) Bromocriptine does not alter growth hormone (GH) responsiveness to GH-releasing hormone in acromegaly. J Clin Endocrinol Metab 62: 601.
296. Hanew K, Sato S, Sasaki A, Shimuzu J, Murakami O, Yoshinaga K (1985) Comparative study on the responses of plasma GH to synthetic GH-releasing factor and other stimulatory and inhibitory agents in patients with acromegaly. Tohoku J Exp Med 145: 161.
297. Shibasaki T, Hotta M, Masuda A, Inaki T, Obara N, Hizuka N, Takano K, Wakabayashi I, Demura H, Ling N, Shizume K (1986) Studies on the response of growth hormone (GH) secretion to GH-releasing hormone, thyrotropin-releasing hormone, and somatostatin in acromegaly. J Clin Endocrinol Metab 63: 167.
298. Losa M, Chiodini PG, Liuzzi A, König A, Müller OA, Schopohl J, von Werder K (1986) Growth hormone-releasing hormone infusion in patients with active acromegaly. J Clin Endocrinol Metab 63: 88.
299. Vance ML, Kaiser DL, Evans WS, Furlanetto R, Vale W, Rivier J, Thorner MO (1985) Pulsatile growth hormone secretion in normal man during a continuous 24-hour infusion of human growth hormone releasing factor (1-40). J Clin Invest 75: 1584.
300. Losa M, Schopohl J, König A, Müller OA, von Werder K (1986) Growth hormone (GH) and prolactin responses to repetitive administration of GH-releasing hormone in acromegaly. J Clin Endocrinol Metab 63: 475.
301. Spada A, Elahi FR, Arosio M, Sartorio A, Guglielmino L, Vallar L, Faglia G (1987) Lack of desensitization of adenomatous somatotrophs to growth-hormone releasing hormone in acromegaly. J Clin Endocrinol Metab 64: 585.

302. Nakagawa K, Akikawa K, Matsubara M, Kubo M (1985) Effect of dexamethasone on growth hormone (GH) response to growth hormone releasing hormone in acromegaly. J Clin Endocrinol Metab 60: 306.
303. Pietschmann P, Schernthaner G, Luger A (1986) Effect of cholinergic muscarinic receptor blockade on human growth hormone (GH)-releasing hormone-(1-44)-induced GH secretion in acromegaly and type I diabetes mellitus. J Clin Endocrinol Metab 63: 389.
304. Hanew K, Sasaki A, Sato S, Goh M, Yoshinaga K (1987) Growth hormone inhibitory and stimulatory actions of L-Dopa in patients with acromegaly. J Clin Endocrinol Metab 64: 255.

Chapter 2.1

SEX DIFFERENCE IN HUMAN GROWTH HORMONE (GH) RESPONSE TO INTRAVENOUS HUMAN PANCREATIC GH-RELEASING HORMONE ADMINISTRATION IN YOUNG ADULTS

A.E.M. Smals, G.F.F.M. Pieters, A.G.H. Smals, Th.J. Benraad*, J.van Laarhoven* and P.W.C. Kloppenborg

Division of Clinical Endocrinology, Department of Medicine, Department of Experimental and Chemical Endocrinology*, University of Nijmegen, The Netherlands.

ABSTRACT

Intravenous administration of a 100 μg dose of human pancreatic growth hormone releasing hormone ($hpGHRH_{1-44}$, indicated by GHRH) disclosed a sex difference in GH responsiveness. The maximum GH increments (41 ± 11 (S.E.M.) vs 15 ± 4 ng/ml, $p^* < 0.05$) and the areas under the curves (419 ± 105 vs 148 ± 53 area units, $p^* < 0.05$) were significantly higher in 12 men than in 10 women. No significant correlation was found in either group between the basal plasma estradiol or testosterone levels and the maximum or integrated GH response to GHRH.

Serum prolactin levels significantly increased in both groups within 5 minutes after GHRH injection (men: $p < 0.001$ vs $t = 0$ and women: $p < 0.05$ vs $t = 0$). The areas under the curves of the prolactin responses (355 ± 184 vs 189 ± 73 area units) and the maximum prolactin increments (58 ± 18 vs 36 ± 6 mU/l, $p^* > 0.10$) were similar.

In conclusion, a sex difference in GH responsiveness to GHRH was found between young adult men and women. Recent in vivo and in vitro data reveal a similar sex difference in rodents and an enhancing effect of androgens, but not estrogens, on the growth hormone response to GHRH. These findings support the theory that in humans testosterone also plays a key role in the genesis of this sex difference.

J Clin Endocr Metab 1986 (62): 336-341

INTRODUCTION

Agents known to cause increase in circulating growth hormone (GH) levels cause more consistent responses in cyclic adult women than in either postmenopausal women (1,2) or men (1,3-5). Significantly higher GH responses to arginine or insulin induced hypoglycemia have been reported in mid- or postcyclic women than in men (4). Merimee et al.(4) demonstrated that administration of estrogens, but not androgens, augmented the arginine induced GH response. Other authors, however, found an enhancing effect of androgens on GH secretion (6,7). In contrast to earlier studies with adult subjects, Pieters et al.(8) reported a two-fold increased GH response in boys as compared to girls after glucose loading.

Thus far, firm evidence supporting a sex difference in GH responsiveness to growth hormone releasing hormone (GHRH) is lacking (9,10). This lack of data prompted us to compare the effect of intravenous GHRH administration on GH in young adult men and women. Furthermore, we assessed whether GHRH elicits a prolactin increase and, if so, whether there is a difference in prolactin responsiveness between sexes.

MATERIALS AND METHODS

Ten normal women (mean age 23.5 $\pm$ 2.4 (S.D.) years, Rohrer index (weight (kg)/height^3(cm) x 10.000) 0.118 $\pm$ 0.01 (normal value 0.113 - 0.130 for women 20-24 year old) and 12 normal men (mean age 23.3 $\pm$ 3.2 years, Rohrer index 0.118 $\pm$ 0.01) (normal value 0.115 - 0.130) were studied. None of the subjects was taking any medication. Six women were studied during the luteal phase of the menstrual cycle, while the remaining four were in the follicular phase. Plasma levels of estradiol were measured to confirm the stage of the menstrual cycle. After an overnight fast, all subjects randomly received 100 µg growth hormone releasing hormone ($hpGHRH_{1-44}$, Bachem, Torrance, CA, U.S.A.) or placebo (saline 0.9%) by i.v. bolus injection at 9.30 a.m. Each subject served as his/her own control. Throughout the test, the subjects stayed fasted and remained in bed. Blood samples for hormone assay were taken via an indwelling venous canula at -60, 45, 30, 15 and 0 minutes before and at +5, 10, 20, 30, 45, 60, 90 and 120 minutes after the GHRH or saline injection.

The GH concentration in plasma was measured by RIA using standards obtained from the Medical Research Council, London U.K. (66/217). The antibody used did not cross-react with thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotrophin (hCG) or human placental lactogen (hPL). Mean interassay coefficients of variation were 13.1%, 12.4% and 12.6% at a mean GH

concentration of 7, 12 and 28 ng/ml, respectively.

Prolactin concentrations in plasma were measured by RIA, using kit-reagents (Immuno Nuclear Corporation; Stillwater, MN). The antiserum had virtually no cross reactivity with GH (0.12%), TSH, hCG, and hPL. Standards were calibrated against MRC Standard 75/504 (i.e. 1 ng/ml Immuno Nuclear Prolactin Standard was equivalent to 20 mIU/l of MRC 75/504). The lower limit of assay detection was 70 mIU/l. The mean interassay coefficient of variation was 6.9% and 7.3% at a mean prolactin concentration of 252 and 1012 mIU/l, respectively.

Testosterone (11) and estradiol (12) were measured by radio-immunoassay with prior chromatographic purification. Plasma cortisol (13) was measured without prior purification. The intra-assay coefficients of variation were 4% (cortisol), 4% (testosterone) and 3% (estradiol).

To avoid interassay variation all samples from a single subject were measured in the same assay.

Areas above baseline of the GH and prolactin response curves were calculated by trapezoidal integration. Statistical analysis was performed using Student's paired (p denoted by p) and unpaired (p*) t-tests, Friedman's non parametric analysis of variance (p**) and Spearman's rank correlation test (p***).

Unless stated otherwise the mean values $\pm$ 1 S.E.M. are given.

RESULTS

SIDE EFFECTS

Mild flushing of the face and chest or a sense of warmth occurred in approximately 50% of the subjects after GHRH injection. No side effects occurred after saline injection.

EFFECT OF I.V. SALINE AND GHRH ON PLASMA GH LEVELS (Table 1, Fig.1 and 2)

The mean basal plasma GH level in men, though slightly higher than in women, did not differ significantly between the 2 groups, before the saline (3.1 $\pm$ 1.1 vs 1.1 $\pm$ 0.2 ng/ml) or GHRH injection (4.3 $\pm$ 1.8 vs 2.2 $\pm$ 0.6 ng/ml) ($p^* > 0.10$).

Saline injection did not alter serum GH levels in either groups (Fig.1). In contrast, within 5 minutes, GHRH significantly increased GH both in men and women ($p < 0.01$) with maximum levels reached within 45 minutes in all but two subjects. Remarkably, the GH response to GHRH at 45 minutes was significantly higher in men than in women (31 $\pm$ 7 vs 10 $\pm$ 4 ng/ml, $p^* < 0.025$), and there was a tendency to higher levels in men than

TABLE 1 Individual plasma GH responses to iv bolus injection of GHRH (100 μg) in 12 men and 10 women at different times [illegible]

	Time (min)												
	−60	−45	−30	−15	0	5	10	20	30	45	60	90	120
Men (n = 12)													
1	4.5	5	3	1.5	<0.5	3.5	1.5	11.5	17	10.5	7.5	2	<0.5
2	<0.5	<0.5	1	1.5	1	5	10.5	14	11.5	9.5	5	4.5	1.5
3	6	6	5.5	10	14	32	40	38	37	32.5	13	5	2.5
4	<0.5	<0.5	<0.5	<0.5	<0.5	1	5	8	6.5	7	21	24.5	
5	<0.5	17.5	30.5	23.5	17	39	83.5	79	106.5	75.5	31	12.5	3.5
6	<0.5	<0.5	<0.5	<0.5	<0.5	9.5	17.5	31.5	43.5	49	84	66.5	25
7	<0.5	<0.5	7.5	14	14	33.5	48	125.5	55	88.5	39	25.5	11.5
8	<0.5	4	3	1	2.5	16	21	35	43	88	41.5	2.5	1.7
9	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	2	4.5	3	7	5	11	<0.5
10	<0.5	<0.5	<0.5	<0.5	<0.5	4.5	14.5	26.5	27	25.5	20	11.5	4.5
11	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	5	8	12	16	18	10.5	3
12	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	8.5	14	15.5	16	20.5	17 ± 5	3.5
Mean ± SEM	1.7 ± 0.6	3.0 ± 1.5	4.5 ± 2.5	5.0 ± 2	4.3 ± 1.8	12 ± 4	23 ± 7	33 ± 10	32 ± 0	35 ± 9	25 ± 6		7 ± 2
Women (n = 10)													
1	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1.5	3	3	4.5	8	8	—
2	9.5	6.5	5	6.5	5	4.5	11.5	16.5	11.5	8	4.5	1	<0.5
3	<0.5	1.5	2	1.5	2	16.5	28	34.5	34.5	28	8.5	13.5	6
4	0.5	<0.5	<0.5	<0.5	<0.5	8	14.5	19	16	14.5	19	17	10.5
5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	5	4	3.5	2.5	3	6	1
6	0.5	3.5	5.5	6	3.5	9.5	18	21.5	15	8.5	5	2.5	<0.5
7	7	5	5	5.5	5	13.0	21	16	23.5	17.5	15.5	5.5	1.5
8	2	1.0	2	1.5	<0.5	<0.5	0.5	1	0.5	1	1	4.5	3.5
9	4	9	15	7	4.5	15.5	15	20	37.5	40.5	39.5	20	6
10	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1.5	5	4.5	4	3	<0.5	<0.5
Mean ± SEM	3.4 ± 1.0	2.8 ± 0.9	3.6 ± 1.4	3.1 ± 1.0	2.2 ± 0.6	7 ± 2	12 ± 3	16 ± 4	15 ± 4	12 ± 4	11 ± 4	7 ± 2	4 ± 2

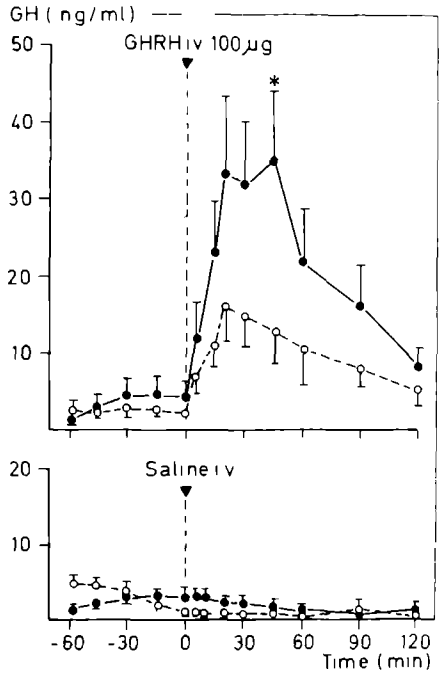


Fig. 1. The mean serum GH responses to i.v. bolus injection of GHRH (100 µg) or saline in 10 healthy young adult women (o-----o) and 12 men (●——●). The asterisk indicates the statistical significance of the differences between men and women at the time of the peak value, *p<0.025.

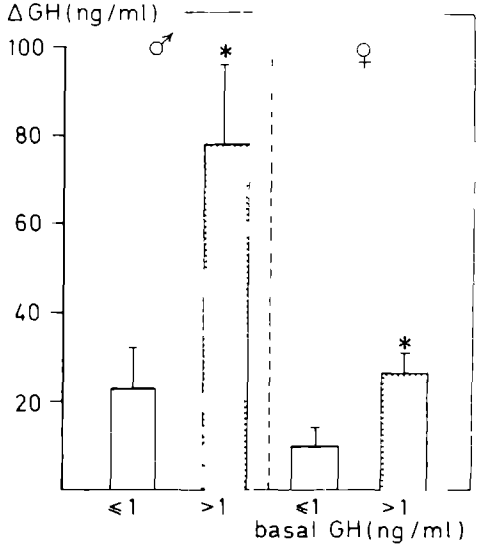


Fig. 2. The mean maximal serum GH responses to i.v. bolus injection of GHRH (100 µg) in 12 healthy adult young men and 10 women in relation to the baseline GH level. The asterisks indicate the statistical significance between the mean maximal GH responses in subjects with baseline serum GH < 1 ng/ml or > 1 ng/ml (*p<0.025).

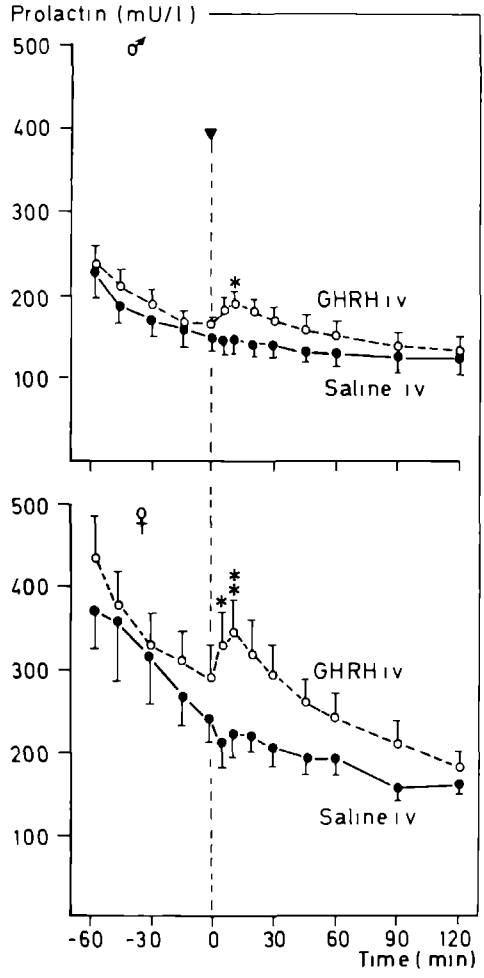


FIG. 3. The mean serum prolactin responses to i.v. bolus injection of GHRH (100 μg) (o-----o) or saline (●——●) in 12 healthy young adult men (upper panel) and 10 women (lower panel). The asterisks indicate the statistical significance between the GHRH and saline curves at the time of the peak value (*$p < 0.05$, **$p < 0.005$).

in women at 30 and 60 minutes. The maximum GH increments (41 ± 11 vs 15 ± 4 ng/ml, $p^* < 0.05$) and the areas under the curves of GH changes (419 ± 105 vs 148 ± 53 area units, $p^* < 0.05$) were also significantly higher in men than in women. The maximum GH responses to GHRH were similar in the follicular and luteal phases of the menstrual cycle (16 ± 6 vs 18 ± 6 ng/ml, $P^* > 0,10$). Taking into account the baseline values and subsequent GH responses to GHRH, subjects with basal GH levels less than 1 ng/ml had lower responses than those with higher baseline GH levels (men 78 ± 18 vs 24 ± 9 ng/ml, $p^* < 0.025$, women 26 ± 4 vs 7 ± 3 ng/ml, $p^* < 0.025$) (Fig.2). In both groups, the number of subjects with basal GH levels exceeding 1 ng/ml did not differ significantly. The mean plasma testosterone levels before the saline and GHRH injections were similar in men (679 ± 76 vs 606 ± 51 ng/100ml). The mean plasma testosterone levels before saline and GHRH in women were significantly different (55 ± 1.9 vs 71 ± 6.8 ng/100ml respectively, $p < 0.05$) before GHRH. The corresponding plasma estradiol levels were 3.5 ± 0.3 vs 3.8 ± 0.2 in men ($p > 0.10$) and 8.6 ± 2.4 vs 10 ± 1.9 ng/100ml in women ($p > 0.10$) (men vs women, $p^* < 0.001$ for saline and GHRH).
No correlation was found in either group between the basal plasma estradiol or testosterone levels and the maximum or integrated GH responses to GHRH ($p^{***} > 0.10$).

EFFECT OF I.V. SALINE AND GHRH ON PLASMA PROLACTIN (Fig.3)

The mean basal plasma prolactin level in women was significantly higher than in men before both the saline and GHRH injection (237 ± 33 vs 153 ± 15 mU/l, $p^* < 0.02$ and 290 ± 36 vs 145 ± 13 mU/l, $p^* < 0.005$ respectively). After i.v. saline injection, plasma prolactin levels significantly decreased ($p^{**} < 0.0005$) in both groups. Within 5 minutes, GHRH injection significantly increased plasma prolactin levels, both in men ($p < 0.001$ vs $t = 0$) and women ($p < 0.05$ vs $t = 0$). Thereafter, prolactin levels remained elevated for 20 - 30 minutes after the injection and then decreased (Fig.3). A statistically significant correlation was not found between the maximum prolactin and GH increments for either group (women $r = -0.20$, men $r = + 0.02$, $p^{***} > 0.10$). The areas under the curves of prolactin changes (355 ± 184 vs 189 ± 73 area units) and the maximum prolactin increments (58 ± 18 vs 36 ± 6 mU/l, $p^* > 0.10$) were similar in both groups.

EFFECT OF I.V. SALINE AND GHRH ON PLASMA CORTISOL (Fig.4)

Plasma cortisol levels significantly decreased in both men and women after saline and GHRH injection ($p^{**} < 0.0005$). No statistically significant difference was found between the basal plasma cortisol levels (men 0.31 ±

0.03 and 0.33 ± 0.04, women 0.34 ± 0.02 and 0.36 ± 0.05 µmol/l, respectively before i.v. saline and GHRH) of the decrements at any time interval throughout the test. Blood glucose levels did not change in both groups throughout both tests ($p^{**} > 0.10$) (data not shown).

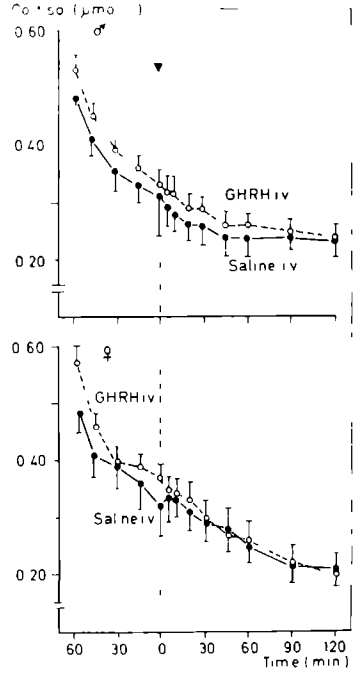


FIG. 4. The mean cortisol responses to i.v. bolus injection of GHRH (o-----o) or saline (●——●) in 12 healthy young men (upper panel) and 10 women (lower panel).

DISCUSSION

Administration of human pancreatic growth hormone releasing hormone evoked different responses in men and women. Young adult men had a maximum and integrated GH response almost two times that of young adult women. The higher GH response to GHRH in men disagrees with earlier studies which described a sex based difference in GH response to stimuli (such as argi-

nine or insulin-induced hypoglycemia) in favour of women. Pretreatment of the men with estrogens, not androgens, abolished this sex difference (1,4,5).

Increased GH responsiveness to GHRH in men as compared to women has not been reported previously. In a meticulous study, Gelato et al.(9) found no sex differences in GH responses to GHRH between 4 groups composed of 5 to 8 men or 5 to 8 women in the midfollicular or midluteal phase, given an i.v. bolus injection of 0.01 to 10 μg/kg GHRH. Japanese investigators reached a similar conclusion (14). Other authors simultaneously administering 4 releasing hormones, including GHRH, found no difference between men and women (10) or a higher response in the women (15). It is difficult to explain why the GH response to GHRH in the present study was almost twice as high in men as in women, despite lower doses of GHRH on a body weight base (1.4 $\pm$ 0.3 vs 1.7 $\pm$ 0.1 μg/kg, $p^* < 0.001$) or per m^2 body surface (0.53 $\pm$ 0.02 μg/m^2 vs 0.58 $\pm$ 0.02 μg/m^2, $p^* < 0.001$) in the men. Since blood glucose levels were similar in both groups, before and during the test, differences in glycemia cannot account for the sex difference (16). In an earlier study (8), a similar sex difference in GH responsiveness to glucose loading was reported between boys and girls tested soon after onset of puberty. This difference could not be accounted for by stress factors, since the tendency for serum GH levels to rise after stress is more pronounced in women than in men (17). In the present study, a relationship was found between basal serum GH levels and the response to GHRH, i.e. higher basal values correlated with higher GH increases. Similarly high basal GH levels were found in both groups. However, the basal GH levels in men were slightly, but not significantly, higher than in women. Moreover, the GH response to GHRH was similar in both the follicular and luteal phases of the menstrual cycle, confirming recent data of Gelato et al.(9) and Evans et al.(18). These authors concluded that the failure to find a phase related difference in GH responsiveness could be construed as evidence against any direct action of estrogens on somatotropes (17). The response difference between men and women, therefore, cannot be attributed to differences in circulating estradiol levels, which were lower in men than in women. Interestingly, a similar sex difference in GH responsiveness to GHRH recently was reported in rats (19,20). Wehrenberg et al.(19) demonstrated that intact 60 day old male rats had a significantly greater GH response to GHRH than female rats. Testosterone treatment greatly enhanced this GH increase both in intact and gonadectomized male rats, whereas estradiol had no such effect in female rats. This sex difference in GH response to GHRH was absent in 30 day old rats. In a preliminary study on GH responsiveness to GHRH in early puberal girls and boys, we also failed to demonstrate a sex difference. Evans et al.(20) recently presented *in vitro* evidence for a gender-dependent difference in

GHRH-stimulated GH release. They further demonstrated that testosterone, not estradiol, enhanced the GHRH-mediated GH release by perifused male rat pituitary cells. Together, the data suggest that androgens may play an important role in modulating the pituitary GH response to GHRH.

In the present study in both men and women a slight, but statistically significant, rise in plasma prolactin was observed. This rise confirms data of Sassolas et al.(21), Borges et al.(22), Pieters et al.(23) and Goldman et al.(24) but contradicts data of most other authors (9,25-27), using doses of GHRH up to 1 μg/kg. No sex difference in prolactin responses could be demonstrated despite higher basal serum prolactin levels in women. The transient rise in these subjects was not an artifact since the prolactin peak occurred before the peak of GH and disappeared before GH reached its peak. Furthermore, cross reactivity of GH in the prolactin assay was minimal (0.12%). Stress factors can be excluded since no rise in prolactin occurred after saline injection. The rise in prolactin levels was slightly but not significantly greater in women than in men. Concomitant secretion of GH and prolactin by GHRH recently was reported in cultured pituitary somatotroph adenoma cells from acromegalic patients (28). Furthermore, in rats the presence of cells secreting both GH and prolactin has been demonstrated in pituitary cultures (29). It is not known if these dually secreting cells are also present in humans or if the lactotrophs possess receptors for GHRH.

The present study presents evidence for the presence of a sex difference in GH responsiveness to GHRH in young adults which cannot be accounted for by differences in circulating estradiol levels. Recent in vivo and in vitro data reveal a similar sex difference in rodents and an enhancing effect of gonadal androgens, not estrogens, on the GH response to its releasing hormone in these animals. These findings support the theory that testosterone may also play a key role in the genesis of this sex difference in humans.

REFERENCES

1. Frantz AG, Rabkin MT 1965 Effects of estrogen and sex difference on secretion of human growth hormone. J Clin Endocrinol Metab 25: 1470.
2. Thompson RG, Rodriques A, Kowarski A, Blizzard RM 1972 Growth hormone: Metabolic clearance rates, integrated concentrations, and production rates in normal adults and the effect of prednisone. J Clin Invest 51· 3193.
3. Glick SN, Roth J, Yalow RS, Berson SM 1966 The regulation of growth hormone secretion. Recent Progress Hormone Research 22: 241.
4. Merimee TJ, Rabinowitz D, Fineberg SE 1969 Arginine-initiated release

of human growth hormone. Factors modifying the response in normal men. New Engl J Med 281: 1434.
5. Merimee TJ, Fineberg SE 1971 Studies of the sex based variation of human growth hormone secretion. J Clin Endocr 33: 896.
6. Martin LG, Clark JW, Connor TB 1968 Growth hormone secretion enhanced by androgens. J Clin Endocrinol Metab 28: 425.
7. Martin LG, Grossman MS, Connor TB, Levitsky LL, Clark JW, Camitta FD 1979 Effect of androgen on growth hormone secretion and growth in boys with short stature. Acta Endocrinol (Copenh) 91: 201.
8. Pieters GFFM, Smals AGH, Kloppenborg PWC 1980 Defective suppression of growth hormone after oral glucose loading in adolescence. J Clin Endocrinol Metab 51: 265.
9. Gelato MC, Pescovitz OH, Cassorla F, Loriaux DL, Merriam GR 1984 Dose-response relationships for the effects of growth hormone releasing factor - (1-44) - NH_2 in young adult men and women. J Clin Endocrinol Metab 59: 197.
10. Hol R, Vink P, Hetzel WD 1985 Combined pituitary stimulationtest with relasing hormones. Acta Endocrinol (Copenh) 108 suppl. 267, Abstract 20, p.18.
11. Smals AGH, Kloppenborg PWC, Lequin RM, Benraad TJ 1976 The effect of gonadotropin releasing hormone on pituitary gonadal function in Klinefelter's syndrome. Acta Endocrinol (Copenh) 83: 89.
12. Smals AGH, Pieters GFFM, Boers GHJ, Raemaekers JMM, Hermus ARMM, Benraad ThJ, Kloppenborg PWC 1984 Differential effect of single high dose and divided small dose administration of human chorionic gonadotropin on Leydig cell steroidogenic desensitization. J Clin Endocrinol Metab 58: 327.
13. Smals AGH, Goverde HJ, Kloppenborg PW, Benraad TJ 1978 Cyproterone acetate and the pituitary adrenal gland function in hirsute women. Acta Endocrinol (Copenh) 87: 352.
14. Takano K, Hizuka N, Shizume K, Asakawa K, Miyakawa M, Hirose N, Shibasaki T, Ling NC 1984 Plasma growth hormone (GH) response to GH-releasing factor in normal children with short stature and patients with pituitary dwarfism. J Clin Endocrinol Metab 58: 236.
15. Debold CR, Sheldon WR, DeCherney GS, Island DP, Thorner MO, Evans WE, Orth DN 1984 Combined anterior pituitary testing with four hypothalamic releasing hormones in normal subjects and patients with pituitary disorders. 7th International Congress of Endocrinology, Quebec, Canada. Abstr 679.
16. Sharp PS, Foley K, Chahal P, Kohner EM 1984 The effect of plasma glucose on the growth hormone response to human pancreatic growth hormone releasing factor in normal subjects. Clinical Endocrinol 20: 497.
17. Daughaday WH 1974 Williams' Textbook of Endocrinology, 5th Edition, W.B. Saunders Company, Philadelphia-London-Toronto, p.53.
18. Evans WS, Borges JCC, Vance ML, Kaiser DL, Rogol AD, Furlanetto R, Rivier J, Vale W, Thorner MO 1984 Effects of human pancreatic growth hormone-relasing factor-40 on serum growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and somatomedin-C concentrations in normal women throughout the menstrual cycle. J Clin Endocrinol Metab 59: 1006.
19. Wehrenberg WB, Baird A, Shao-Yao Ying Ling N 1985 The effects of testosterone and estrogen on the pituitary growth hormone response to growth hormone-releasing factor. Biol Reprod 32: 369.

20. Evans WS, Limber ER, Krieg RJ 1985 Effects of the in vivo gonadal hormone environment on in vitro hGRF 40 stimulated GH release. 67th Annual Meeting Endocrine Society, Baltimore. Abstr 149.
21. Sassolas G, Chatelain P, Cohen R, Boissel JP, Laporte S, Galleyraud J, Claustrat B, Elmcharti A, Chayvialle JA, Cohen H, Ferry S, Underwood L 1984 Effects of human pancreatic tumor growth hormone-releasing hormone ($hpGRH_{1-44}NH_2$) on immunoreactive and bioactive plasma growth hormone in normal young men. J Clin Endocrinol Metab 59: 705.
22. Borges JLC, Gelato MC, Rogol AD, Vance ML, Macleod RM, Loriaux DL, Rivier J, Blizzard RM, Furlanetto R, Evans WS, Kaiser DL, Merriam GR, Spiess J, Vale W, Thorner MO 1983 Effect of human pancreatic tumor growth hormone releasing factor on growth hormone and somatomedin C levels in patients with idiopathic growth hormone deficiency. Lancet II: 119.
23. Pieters GFFM, Smals AGH, Kloppenborg PWC 1984 Responsiveness of growth hormone and prolactin to human pancreatic growth hormone releasing factor in acromegaly, modulatory effect of basal GH levels, effect of concomitant somatostatin administration. First Symposium of The European Neuroendocrine Association, Basle. Abstr E3-10.
24. Goldman JA, Molitch ME, Thorner MO, Rivier J, Vale W, Rechlin SR 1984 Growth hormone releasing hormone 1-40-OH (GHRH) is a prolactin (PRL) releasing factor. 7th International Congress of Endocrinology, Quebec, Canada. Abstr 691.
25. Belchetz PE, Weldon SF, Davis JC, Divez MJ, Smith CS, Harris F 1984 Potential use of human pancreatic growth hormone-releasing factor 1-44 amide. Clin Endocrinol 21: 201.
26. Vance ML, Borges JLC, Kaiser DL, Evans WS, Furlanetto R, Thominet JL, Frohman LA, Rogol AD, MacLeod RM, Bloom S, Rivier J, Vale W, Thorner MO 1984 Human pancreatic tumor growth hormone-releasing factor: dose-response relationships in normal men. J Clin Endocrinol Metab 58: 838.
27. Rosenthal SM, Schriock EA, Kaplan SL, Guillemin R, Grumbach MM 1983 Synthetic human pancreatic growth hormone-releasing factor ($hpGRH_{1-44}$-NH_2) stimulated growth hormone secretion in normal men. J Clin Endocrinol Metab 57: 677.
28. Ishibashi M, Yamaji T 1985 Effects of hypophysiotropic factors on growth hormone and prolactin secretion from somatotroph adenomas in culture. J Clin Endocrinol Metab 60: 985.
29. Frawley LS, Boockfor FR, Hoeffler JP 1985 Identification by plaque assays of a pituitary cell type that secretes both growth hormone and prolactin. Endocrinology 116: 734.

Chapter 2.2

SEX DIFFERENCE IN GROWTH HORMONE RESPONSE TO GROWTH HORMONE RELEASING HORMONE BETWEEN PUBERTAL TALL GIRLS AND BOYS

A.E.M. Smals, G.F.F.M. Pieters, A.G.H. Smals, Th.J. Benraad, P.W.C. Kloppenborg

Division of Clinical Endocrinology, Department of Medicine and Department of Experimental and Chemical Endocrinology (ThJB), University of Nijmegen, Nijmegen, The Netherlands.

SUMMARY

In adult rats and also in young adults a sex difference in GH responsiveness to growth hormone releasing hormone ($GHRH_{1-44}$, indicated by GHRH) has been reported in the literature with the higher responses in males. In young rats, however, the reverse has been found i.e. a higher GH response in females than in males. This discrepancy prompted us to compare GH responsiveness to GHRH in midpubertal tall girls (n=10) and boys (n=8).

Intravenous bolus administration of 100 μg GHRH to these adolescents disclosed a sex difference in GH responsiveness. At all time intervals up to 30 minutes after the bolus the GH responses to GHRH in the girls were significantly higher than in the boys ($P < 0.025$ - $P < 0.05$), whereas the peak GH increments (34 ± 4 vs 19 ± 3 ng/ml, $P < 0.02$) were about twice as high in the former as in the latter.

The data suggest that like in rats, also in humans, sex related changes in pituitary GH sensitivity to GHRH may be an important factor in the pubertal growth and development at least in tall girls and boys.

Acta Endocrinol (Copenh) 1987 (116): 161-164

INTRODUCTION

Recently we reported a difference in growth hormone (GH) responsiveness to growth hormone releasing hormone (GHRH) between young adult women and men with the higher response in the latter (Smals et al.1986). Earlier a similar sex difference had been found in adult rats (90-150 days old) (Evans et al.1985; Wehrenberg et al.1985). In young rats (30 days old), however, the reverse was demonstrated i.e. a higher GH response to GHRH in females than in males (Heiman et al.1984; López et al.1986). These divergent data prompted us to investigate the GH response to a GHRH bolus injection in (tall) midpubertal adolescents.

PATIENTS AND METHODS

Eight pubertal boys and 10 pubertal girls attended our outpatient department because of tall stature. In all of them height exceeded the 90th percentile (P90) of the height distribution in normal children. In 9 of the girls and 4 of the boys height exceeded the 97th percentile.

The study protocol was approved by the hospital ethics committee and informed consent was obtained from all subjects. After an overnight fast all patients received 100 µg GHRH (human pancreatic $GHRH_{1-44}$, Bachem, Torrance, California) by i.v. bolus injection at 09.00 h. The tests were performed with the patients fasting and at bed rest. Blood samples for GH assay were taken via an indwelling intravenous cannula at -30 and 0 minutes before and at 5,10,20,30,60 and 120 minutes after GHRH administration.

The GH levels were determined by specific radio-immunoassay as described earlier (Smals et al.1986) with a coefficient of variation of 13.1%. Testosterone (Smals et al.1976) and oestradiol (Smals et al.1984) were measured by RIA with prior chromatographic purification. The intra-assay coefficients of variation were 4% and 3% respectively.

Statistical analysis was performed using Wilcoxon's two sample test (P denoted by P), Fisher's Chi-square test (P*), Wilcoxon's paired rank test (P**) and Spearman's rank correlationtest (P***).

Unless stated otherwise the mean values ± S.E.M. are given.

RESULTS

CLINICAL CHARACTERISTICS (Table 1)

The groups were comparable in clinical characteristics such as chronologi-

TABLE 1. CHARACTERISTICS OF 8 BOYS AND 10 GIRLS WITH TALL STATURE. THE MEAN VALUES ± S.D. ARE GIVEN

	TALL BOYS	TALL GIRLS	P VALUE
Chronological age (years)	14.8 ± 0.6	14.6 ± 1.8	P = N.S.
Skeletal age (years)	14.6 ± 2.2	13.8 ± 1.4	P = N.S.
Pubertal stage according to Tanner = P4	7 / 8	10 / 10	P*= N.S.
Height above 97th percentile	4 / 8	9 / 10	P*= N.S.
Alkaline phosphatase (U/l)	271 ± 87	166 ± 82	P < 0.02
Menarche present	-	8 / 10	
Plasma testosterone (nmol/l)	12 ± 5.5	-	
Plasma estradiol (nmol/l)	-	0.2 ± 0.04	

N.S. = not statistically significant

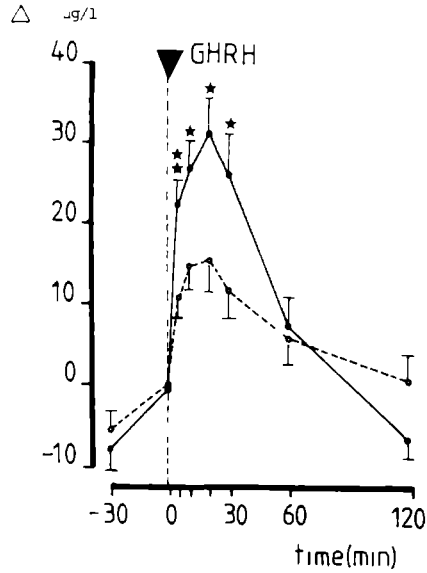


FIG. 1. The mean GH increments to GHRH injection in 10 tall girls (●——●) and 8 tall boys (o----o). The asterisks indicate the statistical significance of the difference in GH levels at the respective time intervals. (*P < 0.05, **P < 0.025).

cal age, skeletal age as determined by the method of Greulich and Pyle (1959) and stage of puberty. As was expected the alkaline phosphatase (AF) levels in the boys were significantly higher than those in the girls.

BASAL GH LEVEL AND GH INCREMENTS AFTER GHRH (Fig.1)

The mean basal GH level at t= 0 minutes did not differ between the boys and girls (6.5 ± 2.0 vs 10 ± 2 μg/l, $P > 0.10$). Five of the 8 boys and 7 out of 10 girls had basal GH levels exceeding 5 μg/l ($P^* > 0.10$). It has to be noted, however, that in both groups the mean GH level at t= 0 min was significantly higher than at t= -30 min ($P^{**} < 0.02$, girls, $P = 0.05$, boys).

After administration of GHRH, GH levels increased in all subjects. At all time intervals up to 30 min after the bolus injection, the mean plasma GH increments (ΔGH) in the girls were significantly higher than in the boys ($P < 0.025$ - $P < 0.05$). Peak GH levels were achieved at 20 min after GHRH administration in both girls (range 5 to 30 min) and boys (range 10 to 60 min) ($P > 0.10$). The mean maximum GH increments were significantly higher in the former than in the latter (34 ± 4 vs 19 ± 3 μg/l, $P < 0.02$). No correlation was found between the basal GH levels and the maximum GH increases after GHRH injection either in the boys (r= +0.29, $P^{***} > 0.10$), or in the girls (r= +0.16, $P^{***} > 0.10$).

It should be noted that the GH profiles after GHRH administration in girls differ from those in the boys: a quicker and higher GH increment is followed by a steep decline, whereas in the boys the decline is more sluggish (mean slope from peak to 120 min 38 ± 4 degrees for the girls vs 23 ± 5 degrees for the boys, $P < 0.05$).

RELATION BETWEEN GH RESPONSES TO GHRH AND BASAL ALKALINE PHOSPHATASE, TESTOSTERONE AND OESTRADIOL LEVELS

Neither in the boys (r= -0.59, $0.05 < P^{***} < 0.10$), nor in the girls (r= +0.43, $P^{***} > 0.10$) was a statistically significant relation found between serum AF levels and the GH increments in response to GHRH. Furthermore, there was no significant relation between basal plasma testosterone levels in the boys (r= +0.25, $P^{**} > 0.10$) or oestradiol levels in the girls (r= +0.04, $P^{***} > 0.10$) and the respective GH increments after GHRH administration.

DISCUSSION

Administration of GHRH disclosed a difference in GH responsiveness between

midpubertal tall boys and girls, the mean GH responses in the latter being about twice as high as in the former. Moreover, in the girls the decline after achieving the maximum was faster. This sex difference was found in the absence of overt differences in clinical characteristics between the two groups, as the mean chronological age, bone age, and the stage of puberty were similar. Only plasma AF levels were significantly higher in the boys than in the girls which is normal for this stage of puberty (Round et al.1973; Pieters et al.1980). No statistical significant correlation was found between AF levels, reflecting growth velocity, and the GH responses to GHRH.

Our finding of a higher GH response to GHRH in midpubertal tall girls than in boys is reminiscent of earlier studies (Sperling et al.1970) reporting a sex difference in GH release after arginine stimulation and are in line with data read from the figures of Gelato et al.(1986), using GHRH testing in normal statured midpubertal adolescents. The finding of Argente et al.(1986) of significantly higher circulating GHRH levels in normal midpubertal girls than in boys may point to a more pronounced hypothalamic drive on GH secretion from the somatotropes in the former, which is also reflected by the reported slightly higher serum somatomedin levels in girls than in boys (Gourmelen et al.1984; Argente et al.1986). It cannot be excluded that stress evoked by testing per se plays a role in the different GH responsiveness to GHRH between girls and boys, as girls are reportedly to be more prone to stress induced GH increases than boys (Daughaday 1974). Indeed in both groups GH levels already increased before the bolus injection of GHRH, but these increments were similar in the girls and the boys.

It should be noted that in the present study the sex difference in GH responsiveness to GHRH was only demonstrated for tall adolescents and therefore may not be pertinent to normally statured girls and boys. It is known that tall adolescents may differ in several aspects from their normally statured peers as they have higher basal 24 hour GH secretory patterns (Albertsson et al.1984; Hindmarsh et al.1986) and significantly higher somatomedin levels (Evain-Brion et al.1983; Gourmelen et al.1984). In the tall adolescents of the present study, the basal plasma GH levels, although rather high, were similar in both groups of girls and boys. Moreover, the latter evinced a similar peak GH response to a GHRH bolus injection as 6 boys with normal stature in the same stage of puberty (9 ± 3 vs 9 ± 7 μg/l, respectively, $P > 0.10$) (Smals et al., unpublished observations). Therefore in our opinion tall stature per se is not likely to be responsible for the sex difference in GH responsiveness between girls and boys. Unfortunately, data on GH responsiveness to GHRH in midpubertal normal girls are lacking.

We have no explanation for the sex difference in GH responsiveness to

GHRH in favour of the (tall) girls, which is completely contrary to the response in young adults, where GH responsiveness is more pronounced in the men. In rats, a similar change in male and female GH responsiveness to GHRH has been reported during pubertal development: in 30 day old rats there is a sex difference in GH responsiveness to GHRH in favour of the females (Heiman et al.1984; Lopez et al.1986). In contrast in adult rats (90-150 days) the GH answer is more pronounced in males than in females owing to the greater proportion of somatotrophs in the pituitary (Leong et al.1985; Ho et al.1986) and their greater sensitivity to GHRH (Ho et al. 1986), probably evoked by the stimulatory effect of testosterone (Evans et al.1985; Wehrenberg et al.1985; López et al.1986).

The data in the present study suggest that like in rats, sex related changes in pituitary GH sensivitity to GHRH may be an important constituent of pubertal development also in humans. Our data urge to prudence in the interpretation of results of GHRH testing which obviously differ according to age, sex, stage of puberty and perhaps the state of mental arousal.

REFERENCES

Albertsson-Wikland K, Rosberg S & Westphal O (1984): 24-h secretory pattern of growth hormone in tall and short children Pediatr Res 18: 102, Abstr 6.

Argente J, Evain-Brion D, Munoz-Villa A, Garnier P, Hernandez M & Donnadieu M (1986): Relationship of plasma growth hormone-releasing hormone levels to pubertal changes. J Clin Endocrinol Metab 63: 680-682.

Daughaday W (1974): Williams' Textbook of Endocrinology, 5th Edn, p.53 W.B.Saunders Company, Philadelphia, page 53.

Evain-Brion D, Garnier P, Schimpff R, Chaussain J & Job J (1983): Growth hormone response to thyrotropin-releasing hormone and oral glucose-loading tests in tall children and adolescents. J Clin Endocrinol Metab 56: 429-432.

Evans W S, Krieg R J, Limber E R, Kaiser D L & Thorner M O (1985): Effects of in vivo gonadal hormone environment on in vitro hGRF-40-stimulated GH release. Am J Physiol, 249: E276-280.

Gelato M C, Malozowski S, Caruso-Nicoletti M et al. (1986): Growth hormone (GH) responses to GH-releasing hormone during pubertal development in normal boys and girls: comparison to idiopathic short stature and GH-deficiency. J Clin Endocrinol Metab 63: 174-179.

Gourmelen M, Bouc Le Y, Girard F & Binoux M (1984): Serum levels of insulin-like growth factor (IGF) and IGF binding protein in constitutionally tall children and adolescents. J Clin Endocrinol Metab 59: 1197-1203.

Greulich W & Pyle S (1959): Radiographic atlas of skeletal development of the hand and wrist. Stanford University Press, Stanford.

Heiman M L, Nekola M V, Murphy W A, Lance V A & Coy D H (1984): Development of prepubertal pituitary responsiveness to growth hormone-releasing factor and luteinizing hormone-releasing hormone. Biol Reprod. Suppl 1:

180. Abstr 299.
Hindmarsh P C, Stanhope R, Kendall B E & Brook C G (1986)· Tall stature: a clinical, endocrinological and radiological study. Clin Endocrinol (Oxf) 25· 223-231.
Ho K Y, Leong D A, Sinha Y N, Johnson M L, Evans W S & Thorner M O (1986)· Sex-related differences in GH secretion in rat using reverse hemolytic plaque assay. Am J Physol 250· E650-654.
Leong D A, Lau S K, Sinha Y N, Kaiser D L & Thorner M O (1985)· Enumeration of lactotropes and somatotropes among male and female pituitary cells in culture· Evidence in favor of a mammasomatotrope subpopulation in the rat. Endocrinology 116· 1371-1378.
López F, González M & Aguilar E (1986)· GH response to GRF 1-29· effects of age, sex, cycle phase and testosterone pretreatment. J Steroid Biochem 25 81S. Abstr 216.
Pieters G F, Smals A G & Kloppenborg P W (1980) Defective suppression of growth hormone after oral glucose loading in adolescence J Clin Endocrinol Metab 51· 265-270.
Round J M (1973) Plasma calcium, magnesium, phosphorus, and alkaline phosphatase levels in normal British schoolchildren. Brit Med J 3· 137-140.
Smals A G, Kloppenborg P W, Lequin R M & Benraad T J (1976)· The effect of gonadotropin releasing hormone on pituitary-gonadal function in Klinefelter's syndrome. Acta Endocrinol (Copenh) 83 829-838.
Smals A G, Pieters G F, Boers G H, Raemaekers J M, Hermus A R, Benraad T J & Kloppenborg P W (1984): Differential effect of single high dose and divided small dose administration of human chorionic gonadotropin on Leydig cell steroidogenic desensitization. J Clin Endocrinol Metab 58· 327-331.
Smals A E, Pieters G F, Smals A G, Benraad T J, Laarhoven van J & Kloppenborg P (1986): Sex difference in human growth hormone (GH) response to intravenous human pancreatic GH-releasing hormone administration in young adults. J Clin Endocrinol Metab 62 336-341.
Sperling M A, Kenny F M & Drash A L (1970)· Arginine-induced growth hormone responses in children· effect of age and puberty. J Pediatr 77· 462-465.
Wehrenberg W B, Baird A, Ying S & Ling N (1985): The effects of testosterone and estrogen on the pituitary growth hormone response to growth hormone-releasing factor. Biol Reprod 32· 369-375.

Chapter 3

HUMAN PANCREATIC GROWTH HORMONE RELEASING HORMONE FAILS TO STIMULATE HUMAN GROWTH HORMONE BOTH IN CUSHING'S DISEASE AND IN CUSHING'S SYNDROME DUE TO ADRENOCORTICAL ADENOMA

A.E.M. Smals, G.F.F.M. Pieters, A.G.H. Smals, Th.J. Benraad*, P.W.C. Kloppenborg

Division of Clinical Endocrinology, Department of Medicine, Department of Experimental and Chemical Endocrinology*, University of Nijmegen, Nijmegen, The Netherlands.

SUMMARY

An absent or severely blunted hGH response to an i.v. bolus injection (100 μg) of human pancreatic growth hormone releasing hormone ($hpGRF_{1-44}$) was found in 7 female Cushing patients (5 with pituitary dependent Cushing's disease and 2 with Cushing's syndrome due to an adrenal adenoma) and 4 men with pituitary dependent Cushing's disease. Three of the female patients and three of the male patients had an adequate hypoglycemia after insulin administration. All these patients showed an absent or blunted hGH response after insulin-induced hypoglycemia. The GHRH data in these patients are in agreement with those in older literature on hGH responsiveness to stimuli like L-Dopa, arginine and insulin-induced hypoglycemia. It is concluded that hypercortisolism inhibits hGH release to various stimuli at the pituitary level.

Clin Endocrinol 1986 (24): 401-407

INTRODUCTION

It has been known for many years that the increase in plasma growth hormone in response to stimuli such as insulin-induced hypoglycemia (Demura et al.1972, Krieger & Luria 1977), arginine (Demura et al.1972), lysine-8-vasopressin (Demura et al.1972) and L-Dopa (Krieger 1973) is impaired in patients with Cushing's disease and Cushing's syndrome, and normalize after correction of the hypercortisolism (Demura et al.1972; Suda et al.1980; Kuwayama et al.1981). So far it is not known whether this defect in growth hormone secretion has its origin in the pituitary or in the hypothalamus. In contrast to the in-vivo defect of growth hormone secretion in endogenous hypercortisolism, in-vitro studies indicate an enhanced growth hormone response to growth hormone releasing hormone (GHRH) after preincubation of cultured pituitary cells with dexamethasone (Vale et al.1983). Similarly, short term administration of dexamethasone to intact and adrenalectomized rats also increases the growth hormone response to GHRH (Wehrenberg et al.1983).

These conflicting data prompted us to investigate the response of human growth hormone (hGH) to GHRH in patients with Cushing's disease (C.D.) and with Cushing's syndrome due to an adrenocortical adenoma (C.S.).

MATERIAL AND METHODS

Approval for the study was obtained from the hospital ethical committee. Seven women (mean age 35 ± 11,7 S.D. year, range 20-51 year) with proven hypercortisolism (5 with C.D. and 2 with C.S.) and 4 men (mean age 37,6 ± 14,6 year, range 17-53 year) with C.D. were studied (Table 1). Twenty-two healthy subjects (12 men, mean age ± S.D. 23,3 ± 3,2 year and 10 women, mean age 23,5 ± 2,4 year) served as controls. Due to the very recently demonstrated sex difference in hGH response to GHRH (Smals et al.1986), the hGH data in the male and female Cushing patients and controls were considered separately. Informed consent was obtained from all patients and control subjects.

At 8.30 a.m. the patients and controls received 100 µg human pancreatic growth hormone releasing factor$_{1-44}$ (Bachem, Torrance, California U.S.A.) by bolus i.v. injection. Blood was collected from an indwelling intravenous heparin lock cannula. Blood samples for hormone assay were collected at -30, 0, 5, 10, 20, 30, 60 and 120 minutes after the injection.

All but one patient also underwent an insulin tolerance test (0,1 - 0,2 U/kg i.v., Actrapid Novo, Copenhagen, Denmark). The hypoglycemic response to the insulin injection was considered adequate when the glucose level

was equal to or less than 2,2 mmol/l and signs of neuroglycopenia were present. Three women and three men had an adequate response according to this protocol.

Plasma hGH and cortisol levels were measured by RIA as reported earlier (Smals et al.1985; Smals et al.1978).

Statistical analysis was performed using Wilcoxon's two sample test. Unless stated otherwise the mean ± 1 S.E.M. are given.

Table 1 Biochemical and pathological data in patients with Cushing's disease (C.D.) and Cushing's syndrome due to adrenal adenoma (C.S.)

Patient no	Sex	Plasma cortisol (μmol/l) Midnight	After overnight 2 mg dexamethasone	After 2 mg dexamethasone four times a day for 2 d	After 4 mg dexamethasone four times a day for 2 d	Clinical diagnosis	PA diagnosis
1	F	0.41	0.28	0.25	0.13	C.D.	Pituitary adenoma
2	F	0.39	0.15		—	C.D.	Pituitary adenoma
3	F	0.46	0.46	0.20	0.07	C.D.	Pituitary adenoma
4	F	0.45	0.37	0.02	0.02	C.D.	Pituitary adenoma
5	F	1.03	0.60	0.49	0.52	C.D.	Pituitary adenoma
6	F	0.52	0.53	0.57	0.58	C.S.	Adrenal adenoma
7	F	0.57	0.50	0.51	0.54	C.S.	Adrenal adenoma
1	M	0.51	0.50	0.16	0.05	C.D.	Pituitary hyperplasia
2	M	0.45	0.35	0.06	0.03	C.D.	Pituitary adenoma
3	M	0.39	0.29	0.03	0.03	C.D.	Pituitary adenoma
4	M	0.44	0.27	0.07	0.09	C.D.	Pituitary adenoma

RESULTS

SERUM hGH RESPONSE TO GHRH (Fig.1 and 2)

The seven women with C.D. or C.S. showed either no rise or only a blunted response of hGH levels after GHRH as compared to the female controls (mean maximum hGH increment 1,7 ± 0,58 vs 15 ± 4 ng/ml, $P < 0.001$). None of the four men with Cushing's disease showed a hGH rise after GHRH, which is in contrast with the clear hGH rise seen in 12 healthy men (mean maximum hGH increment 0 vs 41 ± 11 ng/ml, $P < 0.01$).

SERUM hGH RESPONSE TO INSULIN-INDUCED HYPOGLYCEMIA (Fig.3)

The mean resting glucose level in the 11 patients with C.D. or C.S. (4,5 ± 0,2 mmol/l) did not differ significantly from that in the 22 controls (4,3 ± 0,1 mmol/l).

After insulin administration one female patient with C.D. (patient 1)

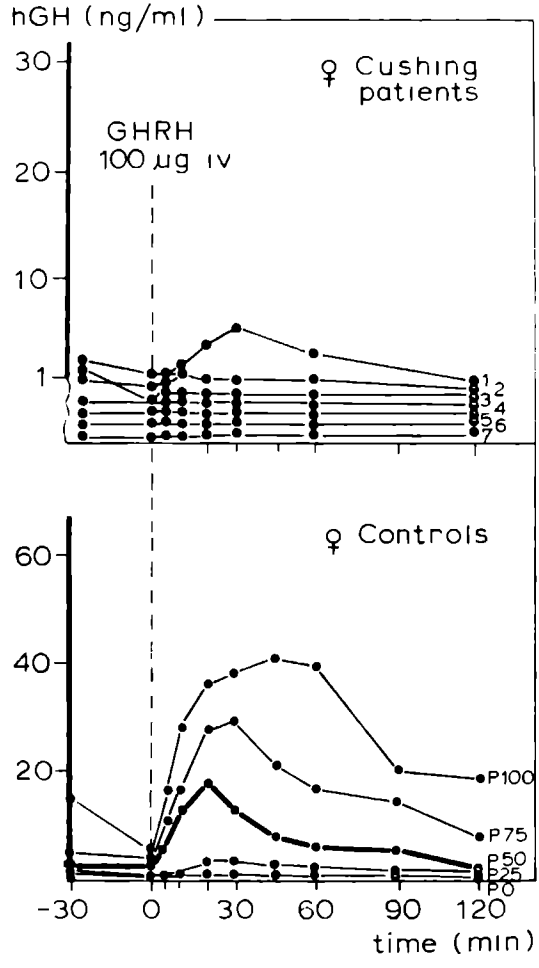


FIG. 1. Response of hGH to GHRH (100 µg i.v.) in 7 women with Cushing's syndrome (patients 1 to 5 with pituitary dependent Cushing's disease and patients 6 and 7 with Cushing's syndrome due to an adrenal adenoma) (upper panel) compared to the response of hGH to GHRH in 10 normal controls (lower panel). The P_0 to P_{100} values are depicted for the subjects.

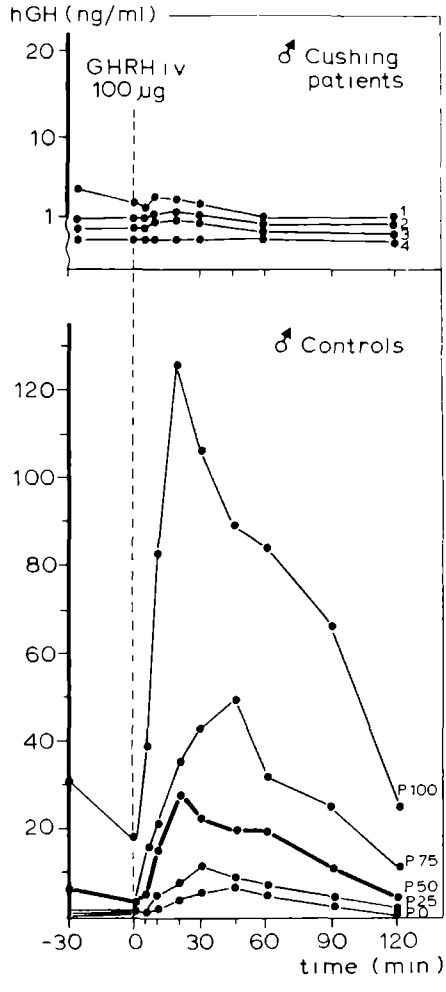


FIG. 2. Response of hGH to GHRH (100 µg i.v.) in four men with pituitary dependent Cushing's disease (upper panel) compared to the response of hGH to GHRH in 12 normal controls (lower panel). The P_0 to P_{100} values are depicted for the control subjects.

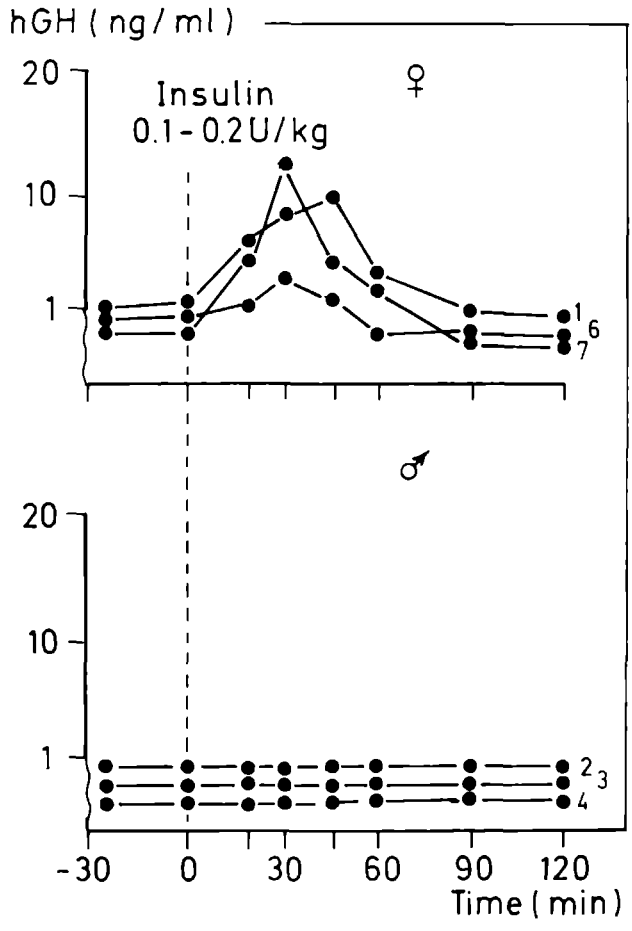


FIG. 3. Response of hGH to i.v. insulin induced hypoglycemia (0,1 - 0,2 U/kg bodyweight) in female Cushing patients (patient 1 with pituitary dependent Cushing's disease and patients 6 and 7 with Cushing's syndrome due to an adrenal adenoma) (upper panel) and male patients with pituitary dependent Cushing's disease (lower panel).

and 2 female patients with C.S. (patients 6 and 7) had an adequate hypoglycemia after insulin administration. Only one patient showed a hGH response that fell within the range found in control subjects (ΔhGH > 10 ng/ml). In the remaining female Cushing's patients the hGH response after insulin induced hypoglycemia was blunted. The 3 men with Cushing's disease who all showed adequate hypoglycaemia did not show any rise of their hGH levels after insulin-induced hypoglycemia.

RESPONSE OF CORTISOL TO GHRH

In contrast with the control subjects in whom plasma cortisol levels significantly fell throughout the test (0,34 ± 0,03 μmol/l at t=0 min to 0,22 ± 0,02 at t= 120 min, $P < 0,001$), in the Cushing patients the levels virtually remained unchanged (0,55 ± 0,06 μmol/l at t=0 min and 0,57 + 0,04 at t= 120 min, $P > 0,10$), due to the absence of the diurnal decrease in plasma cortisol in these patients.

During insulin hypoglycemia plasma cortisol levels also did not change significantly in the patients (basal value 0,57 ± 0,06 μmol/l, peak value 0,55 ± 0,02 μmol/l)

None of the patients with C.D. showed a paradoxical increase of cortisol after the GHRH bolus injection in contrast to the earlier reported paradoxical ACTH and cortisol response after TRH and LHRH (Krieger & Luria 1977; Pieters et al.1982)

No side effects other than a transient flushing in the face and/or upper trunk were noted either in the control subjects or in the patients given GHRH.

DISCUSSION

This study demonstrates for the first time that the response of hGH to GHRH is absent or at least blunted in both male and female patients with C.D. or C.S.

Moreover, our data corroborate findings of Krieger & Luria (1977) and Demura et al.(1972) who reported absent or blunted hGH responses after insulin-induced hypoglycemia in a similar group of patients. The findings of unresponsiveness of hGH to insulin-induced hypoglycemia as well as to GHRH in patients with C.D. and C.S. together strongly suggest that hypercortisolism interferes with hGH release at the pituitary level. However, loss of pituitary responsiveness due to long term GHRH deprivation cannot be excluded as its cause. Recently (Chihara et al.1985) it has been demonstrated that in some patients with idiopathic growth hormone deficiency or a hypothalamic germinoma the blunted (but never absent) hGH response pattern to GHRH could be restored by repetitive intravenous administration of GHRH, suggesting that prolonged deprivation of endogenous GHRH may be the cause of the growth hormone deficiency. We consider that a similar mechanism may be a possible explanation for the blunted hGH response to GHRH in both C.D. and C.S. but have not yet tested this hypothesis.

Our in-vivo results are in sharp contrast to the in-vitro response of growth hormone to GHRH. Vale et al.(1983) demonstrated that pretreatment of cultured rat pituitary cells with dexamethasone enhanced the growth

hormone response to GHRH. In their experiments dexamethasone increased the sensitivity of cultured pituitary cells to GHRH and decreased the sensitivity to somatostatin. In vivo, Wehrenberg et al.(1983) found an enhanced growth hormone response to GHRH in intact and adrenalectomized rats after the administration of dexamethasone during 7 days before the test. It seems possible that only short lasting hypercortisolism enhances the growth hormone response to GHRH, whereas chronic hypercortisolism leads to a blunted response. The data in the present study obviously do not allow us to draw conclusions in this respect. Nakagawa et al.(1985), however, very recently demonstrated that short-term dexamethasone administration (9 mg daily for 2 days) significantly reduced the hGH response to GHRH in six patients with acromegaly, whereas in vitro the monolayer cultured pituitary adenoma cells of three of these patients showed an enhanced hGH release after 2 days of dexamethasone pretreatment. These results were interpreted to mean that in vivo, factors which are so far unknown, override the potentiating effect of dexamethasone on the somatotroph observed in-vitro.

Finally it might be argued that increased blood glucose levels in C.D. and C.S. inhibit the hGH response to GHRH as has been recently demonstrated in control subjects (Sharp et al.1984; Masuda et al.1985) after glucose loading. An argument against this hypothesis is that the basal glucose levels in our patients did not differ significantly from those in controls. From our limited experience we conclude that hypercortisolism inhibits hGH release to various stimuli at the pituitary level.

REFERENCES

Chihara, K., Kashio, Y., Abe, H., Minamitani, N., Kaji, H., Kita, T. & Fujita, T.(1985) Idiopathic growth hormone (GH) deficiency, and GH deficiency secondary to hypothalamic germinoma: effect of single and repeated administration of human GH-releasing factor (hGRF) on plasma GH level and endogenous hGRF-like immunoreactivity level in cerebrospinal fluid. Journal of Clinical Endocrinology and Metabolism, 60, 269-278.

Demura, R., Demura, H., Nunokawa T., Baba, H. & Miura, K. (1972) Response of plasma ACTH, GH, LH and 11-hydroxycorticosteroids to various stimuli in patients with Cushing's syndrome. Journal of Clinical Endocrinology and Metabolism, 34, 852-859.

Krieger, D.(1973) Lack of responsiveness to L-dopa in Cushing's disease. Journal of Clinical Endocrinology and Metabolism, 36, 277-284.

Krieger, D. & Luria, M. (1977) Plasma ACTH and cortisol responses to TRF, vasopressin or hypoglycemia in Cushing's disease and Nelson's syndrome. Journal of Clinical Endocrinology and Metabolism, 44, 361-368.

Kuwayama, A., Kageyama, N., NaKane, T., Watanabe, M. & Kanie, N.(1981) Anterior pituitary function after transsphenoidal selective adenomectomy in patients with Cushing's disease. Journal of Clinical Endocrinology and Metabolism, 53, 165-173.

Masuda, A., Shibasaki, T., Nakahara, M., Imaki, T., Kiyosawa, Y., Jibiki, K., Demura, H., Shizume, K. & Ling N. (1985) The effect of glucose on growth hormone (GH) releasing hormone-mediated GH secretion in man. Journal of Clinical Endocrinology and Metabolism, 60, 523-526.

Pieters, G., Smals, A.G.H., Goverde, H., Pesman, G., Meyer E. & Kloppenborg, P.(1982) Adrenocorticotropin and cortisol responsiveness to thyrotropin-releasing hormone and luteinizing hormone-releasing hormone discloses two subsets of patients with Cushing's disease. Journal of Clinical Endocrinology and Metabolism, 55, 1188-1197.

Sharp, P., Foley, K., Chahal, P. & Kohner, E.(1984) The effect of plasma glucose on the growth hormone response to human pancreatic growth hormone releasing factor in normal subjects. Clinical Endocrinology, 20, 497-501.

Smals, A., Goverde, H., Kloppenborg, P. & Benraad, Th. (1978) Cyproterone acetate and the pituitary adrenal gland function in hirsute women. Acta Endocrinologica (Copenh), 87, 352.

Smals, A.E.M., Pieters, G., Smals, A.G.H., Benraad, Th.J., van Laarhoven, J. & Kloppenborg, P.W.C. (1986) Sex difference in human growth hormone response to i.v. human pancreatic growth hormone releasing hormone administration. Journal of Clinical Endocrinology and Metabolism, 62, 336-341.

Suda, T., Demura, H., Demura, R., Jibiki, K., Tozawa, F. & Shizume, K.(1980) Anterior pituitary hormones in plasma and pituitaries from patients with Cushing's disease. Journal of Clinical Endocrinology and Metabolism, 51, 1048-1053.

Vale, W., Vaughan, J., Yamamoto, J., Spiess, J. & Rivier, J. (1983) Effects of synthetic human pancreatic (tumor) GH releasing factor and somatostatin, triiodothyronine and dexamethasone on GH secretion in-vitro. Endocrinology, 112, 1553-1555.

Wehrenberg, W., Baird, A. & Ling, N. (1983) Potent interaction between glucocorticoids and growth hormone releasing factor in vivo. Science, 221, 556-558.

Chapter 4

STUDIES WITH GHRH IN ACROMEGALY

Chapter 4.1

SEX DIFFERENCE IN THE RELATION BETWEEN SELLAR VOLUME AND BASAL AND GH-RELEASING HORMONE (GHRH) STIMULATED GH IN ACROMEGALY

A.E.M. Smals, G.F.F.M. Pieters, A.G.H. Smals, P.W.C. Kloppenborg

Division of Clinical Endocrinology, Department of Medicine, University of Nijmegen, Nijmegen, The Netherlands.

ABSTRACT

Sellar volume and both basal ($r = +0.54$, $p < 0.02$) and growth hormone releasing hormone (GHRH)-stimulated ($r = +0.41$, $p < 0.05$) growth hormone (GH) levels were directly correlated in a group of 28 acromegalics as were the latter indices ($r = +0.82$, $p < 0.001$). Subdividing the patients according to sex, only in the men a close relation was found between sellar size and both basal ($r = +0.77$, $p < 0.02$) and stimulated GH ($r = +0.71$, $p < 0.02$), not in the women ($r = +0.12$ and $r = +0.02$ respectively, $p > 0.10$).
The presence of a tight relation between sellar volume and basal and GHRH stimulated GH levels in male acromegalics and its complete absence in women are equally intriguing and await further elucidation. A modulating role of gonadal steroids in the genesis of this sex difference remains to be assessed.

Submitted

INTRODUCTION

Sellar enlargement occurs in the majority of patients with acromegaly (Pieters et al.1982; Hanew et al.1987). Jadresic et al.(1982) demonstrated that there is a sex difference in sellar volume between acromegalic women and men, with the greater volume in the latter. Furthermore, a direct relation has been found between basal GH levels and the size of the pituitary adenoma as reflected by the sellar content by most (Jadresic et al. 1982; Wright et al.1969; Klijn et al.1980; De Pablo et al.1981; Hulting et al.1982), but not all authors (Quabbe 1982). No due attention, however, was paid to the sex of the patients. Recently a positive relation between basal GH levels and the GH response to GH-releasing hormone (GHRH) has been demonstrated in these patients (Pieters et al.1984; Losa et al.1985: Pietschmann et al.1986; Smals et al.1987). Such relation was, however, not found by others (Chiodini et al.1985; Gelato et al.1985; Giusti et al. 1985). As the GH answer to GHRH may better reflect activity of the acromegaly (Wood et al.1983) than the basal GH level, we wondered whether there is a more close relation between sellar volume and the GH response to GHRH. In addition we investigated whether there is a sex difference in the relations between these parameters.

MATERIALS AND METHODS

Twenty-eight patients (12 men and 16 women) with active acromegaly (mean age ± (S.D.) 46 ± 13,1 year) participated in this study. Four patients unsuccessfully underwent transsphenoidal surgery. Patient nr 1 in addition underwent irradiation on the pituitary without remission sofar. Informed consent was obtained from all patients after approval of the protocol by the hospital ethics committee. All tests were performed at 9.00 h with the patients fasting and at bed rest. Growth hormone releasing hormone ($hpGHRH_{1-44}$ 100 µg, Bachem Ltd, Torrance, California) was given as a bolus injection. Blood samples for GH assays were obtained via an intravenous canula at -30, 0, 5, 10, 20, 30, 60 and 120 minutes after GHRH injection.

The GH levels were determined by specific radio-immunoassay as described earlier (Smals et al.1986) with an interassay coefficient of variation of 13%. Only the maximal absolute GH increments after GHRH administration are given. Sellar volume was determined according to the method of Di Chiro (1962) (normal values < 1100mm^3). In 6 patients there was suprasellar extension of the adenoma.

Statistical analysis was performed using Spearman's rank correlation test (P) and Wilcoxon's two sample test (P*).

Unless stated otherwise the mean ± S.E.M. is given.

RESULTS (Table 1, Fig.1)

RELATION BETWEEN GH INCREMENTS AFTER GHRH ADMINISTRATION AND BASAL GH LEVELS

The mean basal GH level increased from 47 ± 19 ng/l to a peak level of 220 ± 57 ng/l after the GHRH bolus injection. A striking correlation was found between basal GH levels and the absolute GH increments ($r = +0.82$, $p < 0.001$). After exclusion of the data of the 6 patients with suprasellar extension of their adenoma, the correlation was still statistically significant ($r = +0.75$, $p < 0.001$). In male acromegalics the correlation was $r = +0.84$, $p < 0.01$ ($n=12$) and in the female patients $r = +0.76$, $p < 0.01$ ($n=16$). Similar correlations were found excluding the patients with suprasellar extension of the tumor

RELATION BETWEEN BASAL GH LEVELS AND SELLAR VOLUME

The mean sellar volume in the whole group of acromegalics was 2295 ± 221 mm^3. In 3 patients a normal sellar volume was found. Sellar size was similar in the male and female acromegalics. Sellar volume was not related to age ($r = +0.08$, $p > 0.10$) or to the duration of the disease, neither in the men ($r = +0.14$, $p > 0.10$) nor in the women ($r = +0.04$, $p > 0.10$). A statistically significant correlation could be demonstrated between sellar volume and basal GH level for the whole group of patients ($r = +0.54$, $p < 0.02$). Excluding the six patients with suprasellar extension, the correlation coefficient was $r = +0.53$ ($p < 0.02$, $n=22$). Subdividing the patients according to sex, a close relation between sellar volume and basal GH was only found in the men, not in the women (Fig.1). Exclusion of those patients who showed suprasellar extension virtually did not change these correlations. It has to be noted that the women were older ($p^* < 0.02$) than the men (Table 1).

RELATION BETWEEN ABSOLUTE GH INCREMENTS IN RESPONSE TO GHRH AND SELLAR VOLUME

A statistically significant correlation was found between sellar volume and the maximum GH increments after GHRH administration for the whole group as well as for the group of patients without suprasellar extension of their adenoma ($r = +0.41$, $p < 0.05$, $n=28$ and $r = +0.44$, $p < 0.05$, $n=22$, respectively). Again, subdividing the patients according to sex, only in men a tight relation was found between sellar volume and stimulated GH, not in the women (Fig.1). The data virtually did not change after excluding the patients with suprasellar extension.

TABLE 1. AGE, SEX, SELLAR VOLUME, BASAL GH AND MAXIMUM GH INCREMENTS AFTER GHRH ADMINISTRATION IN 28 PATIENTS WITH ACROMEGALY

PATIENT NR	AGE YRS	SEX	DURATION OF DISEASE (yrs)	SELLAR** VOLUME (mm^3)	BASAL GH (ng/ml)	GH INCREMENTS AFTER GHRH (ng/ml)
→ 1*	28	F	5	1920	230	765
→ 2	25	M	6	6670	142	142
→ 3	61	F	9	4460	100	850
→ 4	36	F	7	2023	75	165
5	57	F	> 1	3308	67	43
6	39	M	15	3200	65	172
7	41	F	9	1800	63	36
8	61	F	6	1853	54	292
→ 9*	21	F	6	2380	36	150
→ 10	36	M	4	1785	34	211
11	38	M	3	2300	29	9
12	45	F	4	2048	28	117
13	62	F	7	2600	22	118
14	66	F	7	2907	22	31
15	65	F	8	2660	22	103
16	42	F	16	1785	20	142
17	36	M	2	1365	19	33
18*	39	M	> 8	1890	14	14
19	50	M	10	1944	4	8
20	31	M	6	599	9	2
21	34	M	7	2707	70	30
22	39	M	3	880	10	5
23	41	F	2	2400	17	15
24	73	F	3	1360	6	5
25*	52	F	> 8	2016	6	2
26	50	M	10	855	10	1
27	47	F	5	1944	100	825
28	41	M	1	2600	37	44

MEAN VALUES:					
MEN:	38±(SD)7	6±(SD)4	2233 ± 465	37 ± 11	100 ± 69
WOMEN:	50± 19.7***	6± 3.5	2341 ± 186	54 ± 14	229 ± 75
ALL:	46± 13.1	6± 3.7	2295 ± 221	47 ± 19	174 ± 50

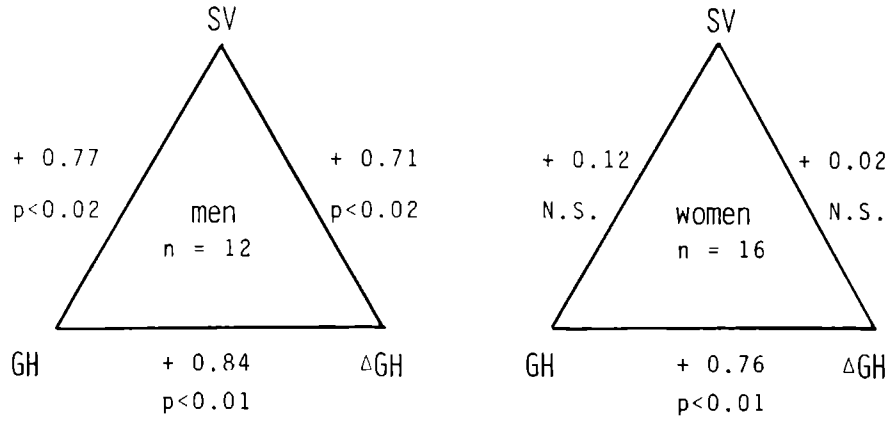
* Patients who underwent unsuccessful transsphenoidal surgery. Patient nr.1 in addition was radiated on the pituitary

** Normal < 1100 mm^3

→ Patients with suprasellar extension

*** $p^* < 0.02$

ALL ACROMEGALICS
(n = 28)



ACROMEGALICS
WITHOUT SUPRASELLAR
EXTENSION
(n = 22)

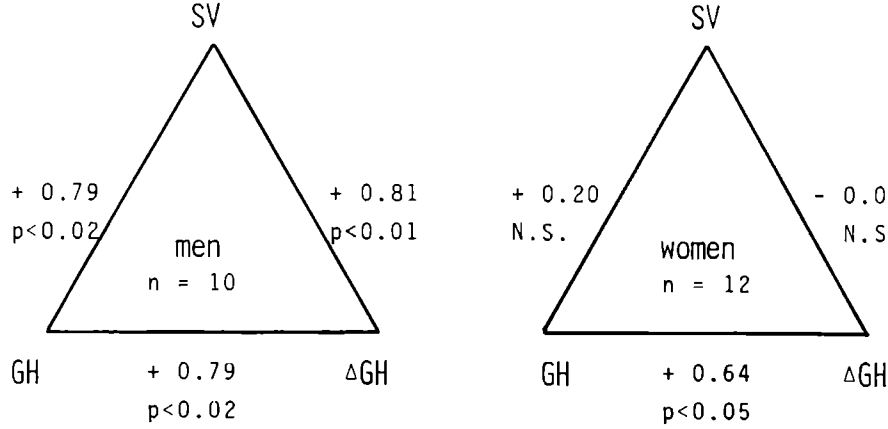


FIG. 1. Spearman's rank correlation coefficients between sellar volume (SV), basal GH and GHRH stimulated GH (ΔGH) in 28 acromegalics (12 men and 16 women) (upper panel). The data for the 22 acromegalics without suprasellar extension of the tumor are given in the lower panel.

DISCUSSION

This study is the first demonstrating a close relation between sellar volume and both basal and GHRH-stimulated GH in male, not however in female acromegalics. Scrutinizing the data of Hulting et al.(1982) we could deduce a similar statistically significant relation between the size of the sella and basal GH levels in their male ($r = +0.52$, $p < 0.05$), but not in their female patients with acromegaly. Similarly analyzing the data of Klijn et al.(1980), again a tight correlation between both parameters was only found in the male acromegalics ($r = +0.79$, $p < 0.001$). A ready explanation for this discrepancy between male and female patients with acromegaly cannot be given. The only difference between male and female acromegalics reported in a large group of patients (Jadresic et al.1982) was the presence of a smaller mean sellar volume in female patients than in men. In our patients no statistically significant differences were found between males and females in sellar volume, duration of the disease, basal GH levels and the presence of suprasellar extension. The mean age of the male acromegalics was, however, significantly lower than that of the female patients, which is in agreement with data of Lawrence et al.(1970) but contrasts with those of Jadresic et al.(1982) This difference in age cannot simply explain the divergent results obtained in male and female patients with acromegaly, unless one expects the well-known protective effects of estrogens (Clemmons et al.1980) to be responsible for a lesser GH effect on target tissues thereby probably delaying the expression of GH excess in women. In this context fits the finding of a tendency for a greater sellar volume in the postmenopausal than in the premenopausal women from this study (cf Table 1). A modulating effect of estrogens on sellar volume therefore remains more than an educated guess. By a similar way of reasoning an enhancing effect of circulating testosterone on tumor growth also cannot be excluded.

Another possible explanation for the lack of a statistically significant correlation between sellar size and both basal and GHRH-stimulated GH levels in women might be that pregnancy in some of them could have led to an increase in pituitary volume or tumor volume which regressed post partum.

Wood et al.(1983) suggested that GH responsiveness to GHRH might be a better indicator of disease activity than GH which is known to be rather poorly correlated with the signs and symptoms of disease activity in acromegaly (Jadresic et al.1982; Quabbe 1980). Therefore an association between GH responsiveness to GHRH - which is determined by the basal GH level - and disease activity of acromegaly is not obvious. Indeed it appears from this study that basal and stimulated GH are equally effective in predicting sellar volume in patients with acromegaly, at least in men.

In summary, our data show that there is a close linkage between sellar volume, basal GH level and GH responsiveness to GHRH, at least in male acromegalics. The presence of these tight relations in men and their absence in women with acromegaly are equally intriguing and await further elucidation. A modulating role of gonadal steroids, if any, in the genesis of this sex difference remains to be assessed.

REFERENCES

Chiodini PG, Liuzzi A, Dallabonzana D, Oppizzi G, Verde GG 1985 Changes in growth hormone (GH) secretion induced by human pancreatic GH releasing hormone-44 in acromegaly: a comparison with thyrotropin-releasing hormone and bromocriptine. J Clin Endocrinol Metab 60: 48.

Clemmons DR, Underwood LE, Ridgway EC, Kliman B, Kjellberg RN, van Wiik JJ 1980 Estradiol treatment of acromegaly. Amer J Med 69: 57.

Di Chiro G, Nelson KB 1962 The volume of the sella turcica. Amer J Roentgenol 87: 989.

Gelato MC, Merriam GR, Vance ML, Goldman JA, Webb C, Evans WS, Rock J, Oldsfield EH, Molitch ME, Rivier J, Vale W, Reichlin S, Frohman LA, Loriaux DL, Thorner MO 1985 Effects of growth hormone-releasing factor on growth hormone secretion in acromegaly. J Clin Endocrinol Metab 60: 251.

Giusti M, Lomeo A, Mazzocchi G, Mignone D, Sessarego P, Monachesi M, Giordano G 1985 The effects of growth hormone-releasing factor (GRF) and dopamine on growth hormone (GH) secretion in acromegaly. J Endocrinol Invest 8: 203.

Hanew K, Sasaki A, Sato S, Goh M, Yoshinaga K 1987 Growth hormone inhibitory and stimulatory actions of L-Dopa in patients with acromegaly. J Clin Endocrinol Metab 64: 255.

Hulting AL, Werner S, Wersäll J, Tribukait B, Anaiko M 1982 Normal growth hormone secretion is rare after microsurgical normalization of growth hormone levels in acromegaly. Acta Med Scand 212: 401.

Jadresic A, Banks LM, Child DF, Diamant L, Doyle FH, Fraser TR, Joplin GF 1982 The acromegaly syndrome. Quart J Med 202: 189.

Klijn JGM, Lamberts SWJ, de Jong FH, van Dongen KJ, Birkenhäger JC 1980 Interrelationships between tumor size, age, plasma growth hormone and incidence of extra sellar extension in acromegalic patients. Acta Endocrinol (Copenh) 95: 289.

Lawrence JH, Tobias CA, Linfoot JA 1970 Successful treatment of acromegaly: Metabolic and clinical studies in 145 patients. J Clin Endocrinol Metab 31: 180.

Losa M, Schopohl J, Stalla GK, Müller OA, von Werder K 1985 Growth hormone releasing factor-test in acromegaly: comparison with other dynamic tests. Clin Endocrinol 23: 99.

De Pablo F, Eastman RC, Roth J, Gorden P 1981 Plasma prolactin in acromegaly before and after treatment. J Clin Endocrinol Metab 53: 344.

Pieters GFFM, Romeijn JE, Smals AGH, Kloppenborg PWC 1982 Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. J Clin Endocrinol Metab 54: 942.

Pieters GF, Smals AE, Hermus AR, Smals AG, Benraad ThJ, Kloppenborg PW 1984 Growth hormone responsiveness to human pancreatic growth hormone releasing factor in acromegaly: modulatory effects of basal hormone levels and of concomitant somatostatin administration. Clin Endocrinol 21: 701.

Pietschmann P, Schernthaner G, Luger A 1986 Effect of cholinergic muscarinic receptor blockade on human growth hormone (GH)-releasing hormone-(1-44)-induced GH secretion in acromegaly and type I diabetes mellitus. J Clin Endocrinol Metab 63: 389.

Quabbe HJ 1980 The endocrinology of growth hormone-secreting microadenomas. In Pituitary Microadenomas (eds. G. Faglia, M.A. Giovanelli & R.M. Macleod), pp. 229-245. Academic Press, London.

Smals AE, Pieters GF, Smals AG, Benraad TJ, Laarhoven van J, Kloppenborg PW 1986 Sex difference in human growth hormone (GH) response to intravenous human pancreatic GH-releasing hormone administration in young adults. J Clin Endocrinol Metab 62: 336.

Smals AE, Pieters GF, Smals AG, Hermus AR, Benraad TJ, Kloppenborg PW 1987 The higher the growth hormone (GH) response to growth hormone releasing hormone (GHRH) in acromegaly, the lower the response to bromocriptine (Br) and thyrotropin releasing hormone (TRH). Clin Endocrinol 27: 43.

Wood SM, Ch'ng JLL, Adams EF, Webster JD, Joplin GF, Mashiter K, Bloom SR 1983 Abnormalities of growth hormone release in response to human pancreatic growth hormone releasing factor (GFR_{1-44}) in acromegaly and hypopituitarism. Br Med J 286: 1687.

Wright AD, McLachlan MSF, Doyle FH, Fraser TR 1969 Serum growth hormone levels and size of pituitary tumour in untreated acromegaly. Br Med J 4: 582.

Chapter 4.2

THE HIGHER THE GROWTH HORMONE (GH) RESPONSE TO GROWTH HORMONE RELEASING HORMONE (GHRH) IN ACROMEGALY, THE LOWER THE RESPONSE TO BROMOCRIPTINE (Br) AND THYROTROPIN RELEASING HORMONE (TRH)

A.E.M. Smals, G.F.F.M. Pieters, A.G.H. Smals, A.R.M.M. Hermus, Th.J. Benraad, P.W.C. Kloppenbrg

Division of Clinical Endocrinology, Department of Medicine, and Department of Experimental and Chemical Endocrinology (ThJB), University of Nijmegen, Nijmegen, The Netherlands.

ABSTRACT

In acromegaly a direct relation has been demonstrated between growth hormone (GH) responsiveness to thyrotropin releasing hormone (TRH) and to the dopaminergic agent bromocriptine (Br). Recent data show an inverse relation between GH responsiveness to Br and to growth hormone releasing hormone (GHRH), but not between the GH responses to GHRH and TRH.

Thirty-one acromegalic patients, 18 women and 13 men (age 46.2 ± 13 years) were studied. Four patients had been treated, but all still had active disease. The GH responses to GHRH ($hpGHRH_{1-44}$, Bachem 100 µg i.v. bolus), TRH (Thyroliberin^R Hoechst 200 µg i.v. bolus) and Br (Parlodel^R 5 mg orally) were assessed in most of the patients. The GH responses to GHRH showed a wide interindividual variation (ΔGH 1-995 ng/ml), which correlated significantly with the basal GH levels ($r= +0.85$, $P<0.0001$, $n=31$). GH increments in response to GHRH were inversely related to the responses to Br, i.e. the lower the GH increase after GHRH the greater the GH decrease after Br ($r= -0.49$, $P<0.01$, $n = 30$). This decrease correlated with the basal prolactin level ($r= +0.45$, $P<0.02$, $n=29$) and also the GH response to TRH ($r= +0.66$, $P < 0.0001$, $n=30$). An inverse correlation was also found between the GH responses to TRH and to GHRH ($r= -0.43$, $P<0.02$, $n=29$).

The data are consistent with the existence of GH secreting adenomas which are more sensitve to GHRH and less to Br and TRH (pure somatotroph adenomas) and of mixed (lactotroph-like) adenomas responsive to TRH and Br but less responsive to GHRH.

Clin Endocrinol 1987 (27): 43-47.

INTRODUCTION

Growth hormone secretion in acromegalic patients with mixed GH and prolactin containing tumours is more sensitive to dopaminergic drugs and TRH than in patients with pure GH-secreting adenomas (Liuzzi et al.1974; Ishibashi & Yamaji 1979,1985; Lamberts et al.1983,1985). Recently Chiodini et al.(1985) and Cozzi et al.(1986) reported that patients responsive to Bromocriptine (Br) have lower GH responses to GHRH than acromegalics which are non-responsive to the dopaminergic agent. This suggested the presence of GH secreting cells with membrane dopamine receptors similar to those on the lactotrophs which respond poorly to GHRH, in the tumours of the Br responders. Such dedifferentiation of the somatotrophs to more primitive lactotrophs would not only make these cells more sensitive to Br but also to TRH. Therefore, if Chiodini's hypothesis were true, GH responsiveness to Br and TRH on the one hand and to GHRH on the other should be inversely related. Such a relationship has been established for GHRH and Br, but not for GHRH and TRH. This work now shows that the GH response to GHRH is inversely related to the GH responses to both Br and TRH, and provides evidence in favour of the hypothesis.

MATERIALS AND METHODS

Thirty-one patients (18 women and 13 men) with active acromegaly (mean age 46.2 ± 13, range 21-73 yr) were studied. Four had previously been treated without success by transsphenoidal pituitary surgery. One patient had had pituitary irradiation. Informed consent was obtained from all patients after approval of the protocol by the hospital ethical committee. All tests were performed in the morning after an overnight fast. Growth hormone releasing hormone ($hpGRF_{1-44}$ 100 μg, Bachem Ltd, Torrance CA) and thyrotropin releasing hormone (Thyroliberin^R 200 μg, Roche Ltd, Basle, Switzerland), were administered in random order on separate days at 09.00 h with the patients remaining in bed. Blood samples for GH and prolactin assays were obtained via an indwelling intravenous canula at -30, 0, 10, 20, 30, 60 and 120 minutes after the releasing hormone. On a separate occasion in the study period a bromocriptine (Br) test was performed, the patients receiving 5 mg Br orally at 8 a.m. Blood for growth hormone assay was sampled immediately before and at 1, 2, 3, 4 and 5 hours after Br ingestion. In patients numbered 8, 12 and 31 the tests were not quite complete.

The hormone levels were measured by specific radioimmunoassays as described earlier with coefficients of variation of 13.1% for GH and of 6.9% for prolactin (Smals et al.1986). Unless stated otherwise the mean

values ± 1 SEM are given. Statistical analysis was performed using Spearman's rank correlation test (P denoted by P).

RESULTS

BASAL GH AND PROLACTIN LEVELS (Table 1)

Basal plasma GH levels showed a wide interindividual variation ranging from 4 to 230 ng/ml (mean 48 ± 9 ng/ml). The basal prolactin levels ranged from 80 to 1739 mU/l (mean 464 ± 74 mU/l). Ten of the patients had prolactin levels higher than 450 mU/l and were arbitrarily considered to be hyperprolactinemic. No statistically significant correlation was found between basal prolactin and GH levels ($r = +0.24$, $P > 0.10$, $n=30$).

GH RESPONSES TO GHRH, TRH AND Br

Plasma GH levels increased in response to GHRH in all patients, but the answer was highly variable both in absolute (Δmax 1 to 995 ng/ml, mean 175 ± 49 ng/ml) and relative terms (10 to 850%, mean 281 ± 43%). A direct relation still was found between the basal GH level and both the absolute and relative peak responses to GHRH ($r = +0.85$, $P < 0.0001$ and $r = +0.44$, $P < 0.02$. respectively, $n=31$). The peak GH responses to TRH ranged from 0 to 1245 ng/ml and from 0 to 2264%.

Br lowered GH levels in all patients (maximum decrease 26 to 95%, mean 68 ± 4%). A statistically significant correlation was found between basal prolactin levels and the relative GH decrease after Br ($r = +0.45$, $P < 0.02$, $n=29$), i.e. the higher the basal prolactin the more pronounced were the relative GH decreases in response to Br.

RELATIONSHIP BETWEEN THE GH RESPONSES TO GHRH, TRH AND Br

In the acromegalic patients a close relationship was found between the percentage of peak increments of GH response to TRH (p TRH) and the decreases in response to Br (pBr) ($r = +0.66$, $P < 0.0001$, $n=30$). The percentage of peak increments to GHRH (pGHRH) and to TRH were inversely correlated ($r = -0.43$, $P < 0.02$, $n=29$). A negative correlation was also found between pGHRH and pBr ($r = -0.49$, $P < 0.01$, $n=30$).

DISCUSSION

This paper is the first to demonstrate the presence of an inverse rela-

tionship between the percentages of peak GH increments to GHRH and to TRH in acromegalic patients, i.e. the higher the GH response to GHRH the lower was the response to TRH. An inverse relation has been established for the GH responses to GHRH and Br (Chiodini et al.1985; Cozzi et al.1986). Chiodini et al.(1985) explained this by suggesting that in acromegalic patients responsive to Br, the GH secreting cells possess membrane dopamine receptors similar to those on lactotrophs, which therefore strongly respond to specific stimuli for prolactin secretion, dopamine and TRH but respond poorly to GHRH. Nevertheless, despite the existence of a close relationship between the GH responses to TRH and Br (Liuzzi et al.1974; Lamberts et al.1983,1985; Chiodini et al.1985), several authors (Chiodini et al.1985; Gelato et al.1985; Losa et al.1985) have failed to demonstrate a statistically significant relationship between the GH responses to GHRH

Table 2. Maximal GH response (Δmax GH) to TRH, GHRH and bromocriptine (Br) and basal GH and prolactin levels in 31 acromegalic patients

Patient no.*	Sex	Age (years)	Δmax GH TRH	Δmax GH GHRH	Δ GH Br	Basal GH (ng/ml)	Basal prolactin (mU/l)
1	M	34	2264	43	−78	70	<80
2	F	50	1275	10	−92	10	992
3	M	39	875	50	−90	10	1063
4	M	38	854	55	−95	29	848
5	F	73	817	83	−78	6	461
6	F	45	446	418	−92	28	1739
7	M	32	433	44	−78	9	129
8	F	65	370	107	—	140	420
9*	M	39	336	100	−73	14	195
10	M	25	319	475	58	142	373
11	F	52	300	33	88	6	493
12*	F	21	216	417	83	36	—
13	F	61	187	541	65	54	183
14	F	41	147	57	95	63	1000
15	F	47	142	825	−51	100	120
16	F	65	138	459	−93	22	200
17	F	66	137	141	−80	22	417
18	F	61	135	850	−71	100	862
19	M	36	93	174	−55	19	146
20	F	42	68	710	−47	20	200
21	M	37	58	620	−36	34	218
22*	F	36	44	220	82	75	862
23*	F	28	42	433	45	230	931
24	M	50	25	175	−57	4	110
25	M	39	20	265	26	65	221
26	F	41	19	88	71	17	274
27	F	62	11	536	−62	22	259
28	M	57	11	64	−37	67	612
29	M	42	9	122	−60	18	173
30	M	51	0	200	−64	8	117
31	F	59	0		−38	7	117

* Patients previously treated by transsphenoidal surgery without success

and TRH. In the discussion of their results Chiodini et al.(1985) state that a dissociation in the response to both stimuli was frequently present in their patients.

The data on the close relationships between GH responsiveness to GHRH, TRH and Br in the present study give strong additional evidence for the hypothesis put forward by Chiodini et al.(1985) and Cozzi et al.(1986) that in GH secreting adenomas, cells sensitive to GHRH and less sensitive to TRH and Br (pure somatotrophs) may coexist with cells responsive to TRH and Br, but less responsive to GHRH (lactotroph-like cells). The wide inter-individual variability of GH responses to the different stimuli (Pieters et al.1984; Chiodini et al.1985; this study) may depend on the degree of dedifferentiation of the somatotroph to lactotroph-like cells (Melmed et al.1983). The recent demonstration of Bassetti et al.(1986) of co-localization of cells secreting only GH, together with mixed cells simultaneously producing GH and prolactin (mammosomatotrophs) in pituitary adenomas of acromegalic patients with hyperprolactinemia, also fits into this concept. Depending on the preponderance in the tumour of the cell types, the GH response to GHRH, TRH or dopamine will vary from more "somatotroph-like" to more "lactotroph-like".

REFERENCES

Bassetti M, Spada A, Arosio M, Vallar L, Brina M & Giannattasio G (1986) Morphological studies on mixed growth hormone (GH) and prolactin (PRL) secreting human pituitary adenomas. Coexistence of GH and PRL in the same secretory granule. Journal of Clinical Endocrinology and Metabolism, 62, 1093-1100.

Chiodini PG, Liuzzi A, Dallabonzana D, Oppizzi G & Verde GG (1985) Changes in growth hormone (GH) secretion induced by human pancreatic GH releasing hormone-44 in acromegaly: a comparison with thyrotropin releasing hormone and bromocriptine. Journal of Clinical Endocrinology and Metabolism, 60, 48-52.

Cozzi R, Dallabonzana D, Oppizzi G, Verde G, Liuzzi A & Chiodini PG (1986) Bromocriptine does not alter growth hormone (GH) responsiveness to GH releasing hormone in acromegaly. Journal of Clinical Endocrinology and Metabolism, 62, 601-604.

Gelato MC, Merriam GR, Vance ML, Goldman JA, Webb C, Evans WS, Rock J, Oldfield EH, Molitch ME, Rivier J, Vale W, Reichlin S, Frohman LA, Loriaux DL & Thorner MO (1985) Effects of growth-hormone releasing factor on growth hormone secretion in acromegaly. Journal of Clinical Endocrinology and Metabolism, 60, 251-257.

Ishibashi M & Yamaji T (1978) Effect of thyrotropin releasing hormone and bromocriptine on growth hormone and prolactin secretion in perfused pituitary adenoma tissues of acromegaly. Journal of Clinical Endocrinology and Metabolism, 47, 1251-1256.

Ishibashi M & Yamaji T (1985) Effects of hypophysiotropic factors on growth hormone and prolactin secretion from somatotroph adenomas in

culture. Journal of Clinical Endocrinology and Metabolisam, 60, 985-993.
Lamberts SW, Klijn JG, van Vroonhoven CC, Stefanko SZ & Liuzzi A (1983) The role of prolactin in the inhibitory action of bromocriptine on growth hormone secretion in acromegaly. Acta Endocrinologica (Copenh), 103, 446.
Lamberts SW, Klijn JG, van Vroonhoven CC & Stefanfo SZ (1985) Different responses of growth hormone secretion to quanfacine, bromocriptine and thyrotropin releasing hormone in acromegalic patients with pure growth hormone (GH) - containing and mixed GH/prolactin - containing pituitary adenomas. Journal of Clinical Endocrinology and Metabolism, 60, 1148-1153.
Liuzzi A, Chiodini PG, Botalla L, Silvestrini F & Muller EE (1974) Growth hormone (GH) releasing activity of TRH and GH-lowering effect of dopaminergic drugs in acromegaly: homogeneity in the two responses. Journal of Clinical Endocrinology and Metabolism, 39, 871-876.
Losa M, Schopohl J, Stalla GK, Muller OA & von Werder K (1985) Growth hormone releasing factor test in acromegaly: comparison with other dynamic tests. Clinical Endocrinology, 23, 99-109.
Melmed S, Braunstein GD, Horvath E, Ezrin C & Kovacs K (1983) Pathophysiology of acromegaly. Endocrine Reviews, 4, 271-290.
Pieters GF, Smals AE, Hermus AR, Smals AG, Benraad TJ & Kloppenborg PW (1984) Growth hormone responsiveness to human pancreatic hormone releasing factor in acromegaly: modulatory effects of basal hormone levels and of concomitant somatostatin administration. Clinical Endocrinology, 21, 701-707.
Smals AE, Pieters GF, Smals AG, Benraad TJ, van Laarhoven J & Kloppenborg PW (1986) Sex difference in human growth hormone (GH) response to intravenous human pancreatic GH releasing hormone administration in young adults. Journal of Clinical Endocrinology and Metabolism, 62, 336-341.

Chapter 4.3

GROWTH HORMONE RESPONSES TO THE RELEASING HORMONES GHRH AND LHRH AND THE INHIBITORS SOMATOSTATIN AND BROMOCRIPTINE IN TRH-RESPONSIVE AND NON-RESPONSIVE ACROMEGALICS

A.E.M. Smals, G.F.F.M. Pieters, A.G.H. Smals, A.R.M.M. Hermus, Th.J. Benraad*, P.W.C. Kloppenborg

Div of Clin Endocrinol, Dept of Med and Dept of Exp and Chem Endocrinol*,- Univ of Nijmegen, The Netherlands

ABSTRACT

In acromegalics, the % peak GH responses to TRH (pTRH) and bromocriptine (pBr) are inversely related with those to GHRH, favouring the hypothesis that in the adenomas of some patients there is a preponderance of GH producing cells with lactotroph-like characteristics, whereas in others pure somatotrophs predominate. The aim of the present study was to investigate whether patients responsive to TRH with allegedly lactotroph-like tumors differ from those patients non-responding to TRH with more somatotroph-like adenomas in their answer to the GH inhibitors Br and somatostatin (SRIF) and the releasing hormones GHRH and luteinizing hormone-releasing hormone (LHRH). The present study demonstrates that the observed reciprocal relations between the GH responses to GHRH, TRH and Br are only present in acromegalics paradoxically responding to TRH (pGHRH vs pTRH -0.73, pGHRH vs pBr -0.60, pTRH vs pBr + 0.54, $p<0.0002$ - $p<0.02$, $n=20$), not in TRH non-responders ($n=10$). In contrast, in these latter patients, not in the former, close relations were found between the % peak GH responses to LHRH (pLHRH) and pGHRH ($r= +0.81$ $p<0.005$) and between pLHRH and the % maximum GH decrements in response to somatostatin (pSRIF) ($r= +0.64$, $p<0.05$). Expectedly the GH response to Br in the TRH responders was significantly higher than in the non-responders (75 ± 4% vs 54 ± 3%, $p^*<0.02$), although it was also substantial in the latter. The GH response to SRIF was remarkedly similar in both groups (64 ± 5 vs 57 ± 9%, $p^*>0.10$). Although the close relations between the GH responses to releasing and inhibiting agents favour the presence of GH-secreting tumours with more somatotroph or more lactotroph characteristics in subsets of acromegalics, they do not effectively predict the therapeutic response to these suppressive agents.

Acta Endocrinol (Copenh) 1987 (116): 53-58

INTRODUCTION

Suppression of growth hormone (GH) by the dopaminergic drug bromocriptine (Br) in acromegalics is the more pronounced the higher the basal prolactin level and the GH response to thyrotropin releasing hormone (TRH) (Liuzzi et al.1974) and the lower its answer to growth hormone releasing hormone (GHRH) (Chiodini et al.1985; Smals et al.1987). The GH responses to the two latter stimuli are inversely related, i.e. the higher the answer to GHRH, the lower the response to TRH (Smals et al.1987). The data have been explained by hypothesizing that in GH secreting adenomas, cells responsive to GHRH and hardly or not to TRH and Br (pure somatotrophs) and cells responsive to TRH and Br but less to GHRH (lactotroph-like cells) coexist (Chiodini et al.1985). Depending on which cell type predominates the GH answer will vary from somatotroph-like to lactotroph-like.

Similarly as has been demonstrated for TRH and Br, a linkage between the GH responses to luteinizing hormone releasing hormone (LHRH) and to somatostatin (SRIF) has been suggested (Hanew et al.1980; Pieters et al.1982; Pieters 1982), whereas there is an antagonism between the effects of SRIF and GHRH on GH secretion (Vale et al.1983; Adams et al.1984; Lamberts et al.1984; Pieters et al.1984a). In view of the hypothesis mentioned above we wondered whether acromegalics responsive to TRH with allegedly lactotroph-like adenomas differ from TRH non-responders with more somatotroph-like tumors, not only in their GH response to Br and GHRH, but also in their answers to somatostatin and LHRH.

MATERIALS AND METHODS

Thirty-one patients (18 women and 13 men) with active acromegaly (mean age 45.8 ± (S.D.) 13, range 21-73 yr) participated in this study. Four of the patients were previously treated without success by transsphenoidal pituitary surgery. One patient in addition underwent pituitary irradiation. Informed consent was obtained from all patients after approval of the protocol by the hospital ethical committee. Growth hormone releasing hormone (hpGRF 1-44 100 μg, Bachem Ltd, Torrance CA), thyrotropin releasing hormone (Thyroliberin^{R} 200 μg, Roche Ltd, Basle Switzerland) and luteinizing hormone releasing hormone (LHRH 100 μg, Hoechst AG, Frankfurt AM, Germany) were administered in random order on separate days at 9 a.m. with the patients fasting and at bedrest. Blood samples for GH and prolactin assays were obtained via an indwelling intravenous canula at -30, 0, 10, 20, 30, 60 and 120 minutes after the administration of the releasing hormone. On separate occasions in the study period, a bromocriptine (Br) and a SRIF test were performed. In the Br test the patients received 5 mg

Br orally at 8 a.m. Blood for GH assay was sampled immediately before and at 1, 2, 3, 4 and 5 hours after Br ingestion. Cyclic somatostatin (SRIF 250 µg, Serono-GmBH, Freiburg, Germany) dissolved in saline was given i.v. for 1 hour at a rate of 300 µg/hour. Blood samples for GH assay were taken immediately before and at 5, 10, 20, 30 and 60 minutes after starting the SRIF infusion. Unfortunately the panel of data was incomplete in 7 patients.

Plasma GH and prolactin levels were determined by specific radio-immunoassay as described earlier (Smals et al.1986).

Using criteria described in the literature (Irie & Tsushima 1972; Schwinn et al.1977; Winkelmann 1977; Schneider et al.1978; Carlson et al. 1984; Pieters et al.1982; Pieters et al.1984b; Tanaka et al.1984; Faglia et al.1985) the GH responses to TRH or LHRH were considered paradoxical if they exceeded the baseline values by 50% or more in at least 2 samples obtained within 30 minutes after the injection.

Statistical analysis was performed using Spearman's rank correlation test (P denoted by P), Wilcoxon's two sample test (P*) and Fisher's Chi Square test (P**).

Unless otherwise stated, the mean + SEM is given.

RESULTS

GH RESPONSES TO GHRH, TRH AND Br (Table 1, Fig.1)

A tendency to a direct relation was found between basal GH and basal prolactin levels ($r= +0.32$, $p < 0.10$, $n=30$). Plasma GH levels in response to GHRH increased in all patients. The absolute and percentage peak increments of GH to GHRH (pGHRH) were highly variable (1-995 µg/l resp. 10 to 850%) but correlated with the basal GH level ($r= +0.85$, $p < 0.0001$ resp. $r= +0.44$, $p < 0.02$). The GH decrease in response to Br was the more pronounced, the higher basal serum prolactin levels ($r= +0.45$, $p < 0.02$, $n=29$). Furthermore, the percentage peak prolactin response to TRH was the higher the lower the basal GH levels ($r= -0.49$, $p < 0.01$, $n=30$) and the higher the percentage peak prolactin response to GHRH ($r= +0.41$, $p < 0.025$, $n=27$). In the whole group of acromegalics, a close relation was found between the percentage peak GH increments to TRH (pTRH) and the decreases after Br administration (pBr) ($r= +0.66$, $p < 0.0001$, $n=30$). pGHRH and pTRH were inversely related ($r= -0.43$, $p < 0.01$, $n=30$). A similar relation was found between pGHRH and pBr ($r= -0.49$, $p < 0.01$, $n=30$).

Table 1

Basal GH and PRL levels in 21 TRH responders and 10 non responders, maximal GH response (Δmax GH%) to TRH, GHRH, GnRH, SRIH and Br and maximal PRL responses (Δmax prolactin %) to GHRH and TRH

Patient No.*	Sex	Age (years)	Basal GH (μg/l)	Basal PRL (mU/l)	Δmax PRL (%) TRH	Δmax PRL (%) GHRH	Δmax GH% TRH	Δmax GH% GHRH	Δmax GH% GnRH	Δmax GH% SRIH	Δmax GH% Br
1	M	34	70	< 80	0	0	2263	13	23	−69	−78
2	F	50	10	992	202	10	1275	10	50	–	−92
3	M	39	10	1063	107	3	875	50	14	−79	−90
4	M	38	29	848	229	27	854	55	5	−71	−95
5	F	73	6	461	321	32	817	83	100	67	−78
6	F	45	28	1739	129	16	146	418	0	68	−92
7	M	32	9	129	210	9	433	44	433	–	−78
8	F	65	110	420	156	20	370	107	43	–	–
9*	M	39	14	195	281	58	336	100	25	−75	−73
10	M	25	142	373	84	8	319	475	22	−38	−58
11	F	52	6	493	54	6	300	83	–	−13	−88
12*	F	21	36	–	–	–	216	417	31	−82	−83
13	F	61	54	183	259	1	187	541	445	−60	−65
14	F	41	68	1000	−2	10	147	57	41	−56	−95
15	F	47	100	120	125	21	142	825	304	−12	−51
16	F	65	22	200	125	6	138	459	30	−81	−93
17	F	66	22	417	47	11	137	141	0	−29	−80
18	F	61	100	862	108	12	135	850	36	−92	−71
19	M	36	19	146	191	32	93	174	0	−90	−55
20	F	42	20	200	400	41	68	710	452	−84	−47
21	M	37	34	218	111	24	58	620	7	−80	−36
22*	F	36	75	862	125	–	44	220	13	− 8	−82
23*	F	28	230	931	92	32	42	433	64	−83	−45
24	M	50	4	110	267	24	25	175	20	−50	−57
25	M	39	65	221	129	16	20	265	76	−75	−26
26	F	41	17	274	216	10	19	88	28	−53	−71
27	F	62	22	259	184	7	11	536	444	−75	−62
28	M	57	67	612	119	10	11	64	17	−69	−37
29	M	42	18	173	91	12	9	122	18	−58	−60
30	M	51	8	117	419	–	0	200	143	−89	−64
31	F	59	7	117	287	–	0	–	0	−10	−38

* Patients previously treated by transsphenoidal surgery without success. Patient No. 23 also underwent unsuccessful pituitary irradiation.

GH RESPONSES TO GHRH, TRH AND Br IN RELATION TO TRH RESPONSIVENESS

According to their GH answer to TRH, the acromegalics could be subdivided in those paradoxically responding to TRH (n=20) and those non-responding (n=10). The two groups did not differ significantly from each other, either in age (45.6 ± (S.D.) 16.0 vs 46.5 ± 11.0 year, $p^* > 0.10$), or in sex distribution (males 38 vs 40%, females 61 vs 60%, $p^{**} > 0.10$), or in duration of disease (7.15 + (S.D.) 3.7 vs 6.2 ± 4.5 year, $p^* > 0.10$). The GH decrements in response to Br, however, were more pronounced in the former group than in the latter (74.9 ± 4.4 vs 54 2 ± 5.5%, $p^* < 0.025$).

Only in the TRH-responder group - not in the TRH non-responders - were statistically significant correlations found between pGHRH and pTRH ($r= -0.73$), between pTRH and pBr ($r= +0.54$) and between pBr and pGHRH ($r= -0.60$) (Fig.1). Furthermore, only in this group were the basal serum prolactin levels and the GH responses to Br directly correlated ($r= +0.60$, $P < 0.02$). Moreover, only in the TRH-responders were the prolactin responses to TRH and GHRH positively correlated ($r= +0.54$, $p < 0.02$, n=20).

GH RESPONSES TO GHRH, LHRH AND SRIF

Ten of the 30 patients tested showed an increase of their GH levels after LHRH, exceeding 50% of the baseline value (range 50 to 445%). In the whole group of acromegalics, no statistically significant correlation was found between the % GH increments in response to GHRH and to LHRH (pLHRH) ($r= +0.25$, $p > 0.10$, n=30).

During SRIF infusion, plasma GH levels declined in all patients to nadir values -7 to -92% (mean -61 ± 5%). No statistically significant correlation was found between the % GH decrements in response to SRIF (pSRIF) and the % increments after either GHRH ($r= +0.27$, n=27, $p > 0.10$) or LHRH ($r= +0.25$, n=27, $p > 0.10$).

GH RESPONSES TO GHRH, LHRH AND SRIF IN RELATION TO TRH RESPONSIVENESS (Table 1, Fig.1)

The median GH response to GHRH in the TRH-responder group was 140% (range 10 -850%), in the TRH-non-responders 210% (range 64-536%). A paradoxical GH answer to LHRH was observed in 6 out of 20 of the former patients and 4 of the latter ($p^{**} > 0.10$). GH decrements in response to SRIF were similar in the TRH-responders and non-responders (64 ± 5 vs 57 ± 9%, $p^* > 0.10$).

In the TRH-responder group, no statistically significant correlations were present between the % GH answers to SRIF, GHRH or LHRH. In the non-responder group, however, a tight relation was found between the GH re-

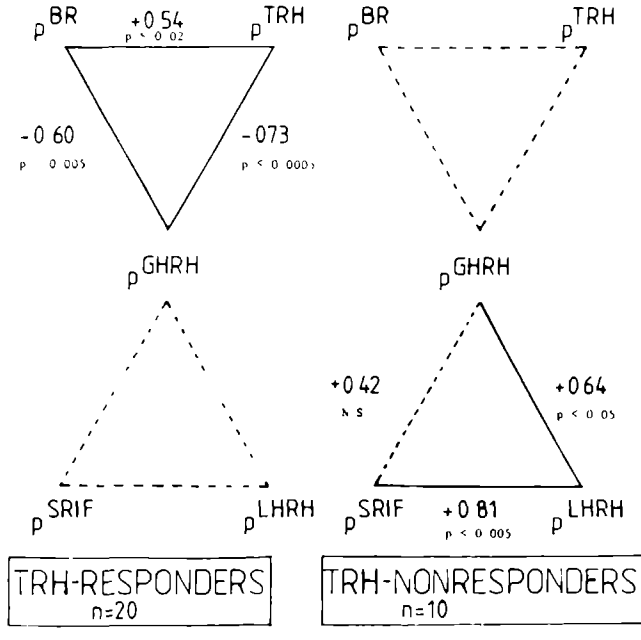


FIG. 1. Relationship between the percentage GH responses to GHRH (pGHRH), TRH (pTRH) and Br (pBr) (upper triangles) and between the answers to GHRH, LHRH (pLHRH) and SRIF (pSRIF) (lower triangles) in TRH responsive (Δ max GH% > 50) and non-responsive acromegalics. Statistically significant correlations are indicated by heavy lines, not significant correlations by dashed lines.

sponses to SRIF and LHRH (r= +0.81) and between those to LHRH and GHRH (r= +0.64). The correlation between pGHRH and pSRIF lacked statistical significance (r= +0.42).

DISCUSSION

In acromegaly, a direct relation has been demonstrated between the GH suppressive effect of Br and both the basal prolactin level and the GH response to TRH (Liuzzi et al.1974; Smals et al.1987). Furthermore an inverse relation was found between the GH responses to Br and to GHRH (Chiodini et al.1985; Smals et al.1987) and finally, also between the answers to GHRH and TRH (Smals et al.1987). The present study extends these data, demonstrating that the close relations between the GH responses to the stimulatory and inhibitory agents only hold true for those acromegalic patients presenting a paradoxical GH increase in response to TRH, not for the TRH-non-responders. The data favour the hypothesis put forward by Chiodini et al.(1985) that depending on the preponderance of the cell type in the adenoma of these acromegalics, the GH answers to GHRH, TRH and Br will vary from "lactotroph-like" to "somatotroph-like". Indeed GH suppression by Br in the patients of the present study was more pronounced in the TRH-responders. Also in line with the hypothesis is the presence of a direct relation between basal prolactin and GH response to Br at least in the TRH-responders, and the inverse relation between basal GH and the answer of prolactin to TRH. Furthermore, only in the TRH-responding acromegalics were the prolactin responses to TRH and GHRH highly correlated. The data of Losa et al.(1985) of a linkage between the TRH-induced GH rise and the GHRH-mediated prolactin increase also fit in a forementioned concept.

Similarly as has been demonstrated for TRH and Br, a linkage between the GH stimulating effect of LHRH and the inhibitory effect of SRIF has been suggested in the literature (Pieters 1982; Pieters et al.1982). Furthermore, there is the well-known antagonism between SRIF and GHRH in their action on GH (Vale et al.1983; Adams et al.1984; Lamberts et al.1984; Pieters et al.1984a). From the present study it appears that in the TRH non-responsive acromegalics - not in the responders - there is a close relation between the GH responses to LHRH and to SRIF and between those to LHRH and to GHRH. Together the data suggest that in acromegalics with supposed mixed mammosomatotroph adenomas TRH predicts the GH responses to GHRH and Br, whereas in the more somatotroph-like tumors LHRH presages GH responsiveness to LHRH and SRIF. Considering, however, the GH responses to the suppressive agents in both subgroups of acromegalics it appears that GH suppression by Br is admittedly more pronounced in the TRH-responders,

but is still substantial in the non-responders. This suggests that also in these patients dopamine-sensitive GH secreting cells are present. Furthermore, it has to be noted that GH suppression by SRIF is almost identical in both groups of acromegalics, whereas such effect would be expected to be more pronounced in those acromegalics not responding to TRH.

Summarizing, the data illustrate that although there may be close relations between the GH responses to releasing and inhibiting agents favouring a more "lactotroph-" or "somatotroph-like" origin of GH overproduction in subgroups of acromegalics, these do not yet allow the drawing of firm conclusions with respect to the choice of therapy in individual patients.

REFERENCES

Adams EF, Bhuttacharji SC, Halliwell CLJ, Loizou M, Birch G & Mashiter K (1984): Effect of pancreatic growth hormone releasing factors on GH secretion by human somatotrophic pituitary tumors in cell culture. Clin Endocrinol 21: 709-718.

Carlson HE, Levin SR, Braunstein GD, Spencer EM, Wilson SE & Hershman JM (1984): Effect of bromocriptine on serum hormones in acromegaly. Horm Res 19: 142-152.

Chiodini PG, Liuzzi A, Dallabonzana D, Oppizzi G & Verde G (1985): Changes in growth hormone (GH) secretion induced by human pancreatic GH releasing hormone-44 in acromegaly: a comparison with thyrotropin releasing hormone and bromocryptine. J Clin Endocrinol Metab 60: 48-52.

Faglia G, Arosio M & Ambrosi B (1985): Recent advances in diagnosis and treatment of acromegaly. *In: Imura H (ed.). The pituitary gland*, pp 363-404, Raven Press, New York.

Hanew K, Kobukun M, Sasaki A, Mouri T & Yoshinaga K (1980): The spectrum of pituitary hormone responses to pharmacological stimuli in acromegaly. J Clin Endocrinol Metab 51: 292-297.

Irie M & Tsushima T (1972): Increase of serum growth hormone concentration following thyrotropin-releasing hormone injections in patients with acromegaly or gigantism. J Clin Endocrinol Metab 35: 97-100.

Lamberts SWJ, Verleun T & Oosterom R (1984): The interrelationship between the effects of somatostatin and human pancreatic growth hormone-releasing factor on growth hormone release by cultured pituitary tumor cells from patients with acromegaly. J Clin Endocrinol Metab 58: 250-254.

Liuzzi A, Chiodini PG, Botalla L, Silvestrini F & Müller EE (1974): Growth hormone (GH)-releasing activity of TRH and GH-lowering effect of dopaminergic drugs in acromegaly: Homogeneity in the two responses. J Clin Endocrinol Metab 39: 871-876.

Losa M, Schopohl J, Müller OA & von Werder K (1985): Growth hormone releasing factor induces prolactin secretion in acromegalic patients but not in normal subjects. Acta Endocrinol (Copenh) 109: 467-473.

Pieters GFFM, Romeyn JE, Smals AGH & Kloppenborg PWC (1982): Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocryptine in acromegaly. J Clin Endocrinol Metab 54: 942-948.

Pieters GFFM (1982): Paradoxical responses to releasing hormones. Thesis Nijmegen: page 95-97.

Pieters GFFM, Smals AEM, Hermus ARMM, Smals AGH, Benraad ThJ & Kloppenborg PWC (1984a) Growth hormone responsiveness to human pan-creatic growth hormone releasing factor in acromegaly: modulatory effects of basal hormone levels and of concomitant somatostatin administration. Clin Endocrinol 21: 701-707.

Pieters GFFM, Hermus ARMM, Smals AGH & Kloppenborg PWC (1984b): Paradoxical responsiveness of growth hormone to corticotropin-releasing factor in acromegaly. J Clin Endocrinol Metab 58: 560-562.

Schneider KR, Müller G, Wiegelmann W, Solbach HG & Krüskemper HI (1978): Vergleichende Untersuchungen zur Wachstumhormonsekretion bei Akromegalie nach isolierter und kombinierter Anwendung des Insulinhypoglykäemie, LH-RH und TRH-tests. Klin Wschr 56: 461-467.

Schwinn G, Dirks H, McIntosh C & Köbberling J (1977): Metabolic and clinical studies on patients with acromegaly treated with bromocriptine over 22 months. Eur J Clin Invest 7: 101-107.

Smals AEM, Pieters GFFM, Smals AGH, Benraad TJ, Laarhoven van J & Kloppenborg PWC (1986): Sex difference in human growth hormone (GH) response to intravenous human pancreatic GH-releasing hormone administration in young adults. J Clin Endocrinol Metab 62: 336-341.

Smals AEM, Pieters GFFM, Smals AGH, Hermus ARMM, Benraad ThJ & Kloppenborg PWC: The higher the growth hormone (GH) response to growth hormone releasing hormone (GHRH) in acromegaly, the lower the response to bromocriptine (Br) and thyrotropin releasing hormone (TRH). Clin Endocrinol (Oxf) 27: 43-47.

Tanaka K, Watabe T, Yoshida H & Shimiza N (1984): Effect of synthetic ovine corticotropin-releasing factor on growth hormone secretion in patients with acromegaly. Endocrinol Japon 31: 355-361.

Vale W, Vaughan J, Jamamoto G, Spiess J & Rivier J (1983): Effects of synthetic human pancreatic (tumor) GH releasing factor and somatostatin, triiodothyronine and dexamethasone on GH secretion in vitro. Endocrinology 112: 1553-1555.

Winkelmann W (1977): Plasma growth hormone response to TRH, LH-RH and bromocriptine in patients with acromegaly. Acta Endocrinol (Copenh) Suppl 208: 26 Abstr.

Chapter 4.4

GROWTH HORMONE RESPONSIVENESS TO HUMAN PANCREATIC GROWTH HORMONE RELEASING FACTOR IN ACROMEGALY: MODULATORY EFFECTS OF BASAL HORMONE LEVELS AND OF CONCOMITANT SOMATOSTATIN ADMINISTRATION

G.F.F.M. Pieters, A.E.M. Smals, A.R.M.M. Hermus, A.G.H. Smals, Th.J. Benraad* and P.W.C. Kloppenborg

Department of Medicine, Division of Endocrinology, and Department of Experimental and Chemical Endocrinology*, St.Radboud Hospital, University of Nijmegen, Nijmegen, The Netherlands.

SUMMARY

Human pancreatic growth hormone releasing factor 1-44 (hpGRF), 100 μg was administered as an i.v. bolus injection to eleven patients with acromegaly. The mean serum growth hormone (GH) levels rose ($p<0.001$) from 54 ± 20 ng/ml to 215 ± 126 ng/ml (± SEM) 20 min after the injection. Although the maximum response of GH levels was highly variable it correlated positively with the individual GH levels ($p<0.01$, $R_S = +0.80$). Thus the higher the GH levels, the greater the responsiveness to hpGRF. Administration of somatostatin (SRIF), 300 μg/h, lowered basal GH levels from 76 ± 38 ng/ml to 13 ± 5 ng/ml ($p<0.01$) after 1 h. hpGRF administration during concomitant SRIF infusion also led to highly variable growth hormone responses. The maximum GH responses again correlated positively with the GH levels before hpGRF after 1 h of SRIF administration ($p<0.05$, $R_S = +0.79$). GH responses to hpGRF were completely blocked by SRIF in three out of four patients whose GH levels decreased to normal levels during SRIF infusion. Our data illustrate that the pituitary in acromegaly is normally responsive to both SRIF and hpGRF but at a higher setting of basal GH levels.

Clin Endocrinol 1984 (21): 701-707

INTRODUCTION

After the isolation, purification and subsequent synthesis of a growth hormone (GH) releasing factor (from pancreatic tumors causing acromegaly by GH cell hyperplasia of the pituitary; Guillemin et al.1982; Rivier et al.1982), several investigators have shown the specific GH-releasing activity of the human pancreatic growth hormone releasing factors (hpGRF 1-40 and hpGRF 1-44) in laboratory animals. These peptides appeared to be devoid of action on the secretion of the other anterior pituitary hormones (Guillemin et al.1982; Rivier et al.1982; Vale et al.1983; Wehrenberg et al.1982a,b). In healthy men, hpGRF 1 μg/kg, or 100 μg per subject, i.v., specifically stimulated GH release to a highly variable degree (maximal responses from 2 to 80 ng/ml) (Thorner et al.1983; Wood et al.1983; Rosenthal et al.1983; Gelato et al.1983a). In acromegaly, hpGRF also specifically stimulated GH release in vivo (Wood et al.1983; von Werder et al.1983; Gelato et al.1983b; Shibashaki et al.1984), as well as in surgically removed pituitary tumour tissue in vitro (Adams et al.1983a,b; Webb et al.1983; Lamberts et al.1984). So far, the effect of concomitant administration of hpGRF and somatostatin (SRIF) has only been reported from studies in vitro, in rat pituitary tissue (Vale et al.1983; Harwood & Grewe 1983) and in pituitary tissue from patients with acromegaly (Adams et al.1983a,b; Lamberts et al.1984). In the latter investigations coincubation of the culture medium with high doses of SRIF completely blocked the stimulatory effect of hpGRF in the acromegalic tissue. In the rat pituitary tissue SRIF did not block hpGRF-induced GH release completely (Vale et al.1983). From similar in vitro experiments Brazeau et al.(1983) concluded that the interaction between hpGRF and SRIF at the pituitary level was non-competitive. We report observations of hpGRF-induced blood GH patterns in patients with acromegaly in the absence and in the presence of exogenous SRIF.

METHODS

Eleven patients with acromegaly (five men of 41 ± 7 (SD) years and six women of 48 ± 15 years) were studied. Five patients were temporarily treated with bromocriptine. This treatment was discontinued at least 2 weeks before the start of this investigation. The remaining six patients had never previously been treated for acromegaly. Clinical and biochemical data of these patients are given in Table 1. Informed consent was obtained from all patients. hpGRF 1-44 was purchased from Bachem (Torrance, California, USA) One mg of the peptide was dissolved in sterile 1 mmol/l hydrochloric acid, 1 mmol/l ascorbic acid and 154 mmol/l bacteriostatic

sodium chloride containing 10% (w/v) mannitol (USP) and 0.25% human serum albumin (USP), giving a final concentration of 100 μg hpGRP 1-44/ml. This solution was then sterilized by filtration. Sterility was confirmed by negative culture of bacteria. The pyrogen free solution (Pyrogentest, Mallinckrodt) was placed in sterile vials and stored at-56°C. One vial (100 μg) per patient was thawed immediately before administration at 0900 h with the patients fasting and at bedrest. Blood samples for hormone assay were collected at -30, 0, 5, 10, 20, 30, 60 and 120 min via an indwelling intravenous cannula kept open with a diluted (10%) solution of heparin. After an interval of 2-14 days, cyclic somatostatin 1-14 750 μg (Serono-GmBH, Freiburg, Germany) dissolved in 75 ml saline was given intravenously, using a micro-infusion pump at a speed of 30 ml/h (300 μg/h) for 2 h. After 60 min of SRIF infusion, 100 μg of hpGRF was administered as a bolus injection. Blood samples for hormone assay were collected at regular intervals before and during the first hour of SRIF administration and at 0, 5, 10, 20, 30 and 60 min after the bolus injection of hpGRF. Plasma GH (coefficient of variation, CV, 15%) was determined by a specific radioimmunoassay as described previously (Smals et al.1978). Statistical analysis was performed using Wilcoxon's paired rank test (p values denoted by p). Friedman's non-parametric analysis of variance (p= p^X) and Spearman's rank correlation test (correlation coefficient: R_S, p= p^{XX}). Unless otherwise stated, the mean values ± 1 SEM are given. Sellar volume was determined according to the method of Di Chiro & Nelson (1962).

Table 1. Clinical and biochemical data on the 11 patients with acromegaly

Patient no	Sex	Age (years)	Sellar volume mm³ *	Duration of disease (years)	GH during OGTT (ng/ml) 0	GH during OGTT (ng/ml) 60	Previous treatment
1	f	27	3622†	4	385	345	–
2	f	62	3808†	>15	156	202	Bromocriptine
3	f	55	3302	> 5	80	50	
4	m	36	1606	3	92	44	
5	m	37	4180	4	32	28	
6	f	45	1590	> 5	54	31	Bromocriptine
7	f	36	2736	>10	42	37	Bromocriptine
8	f	64	2660	12	27	20	Bromocriptine
9	m	35	847	> 5	26	15	—
10	m	49	1352	9	—		Bromocriptine
11	m	48	1063	>10	5	3	

* Normal value < 1100 mm³

† With suprasellar extension of the tumour and visual field defects

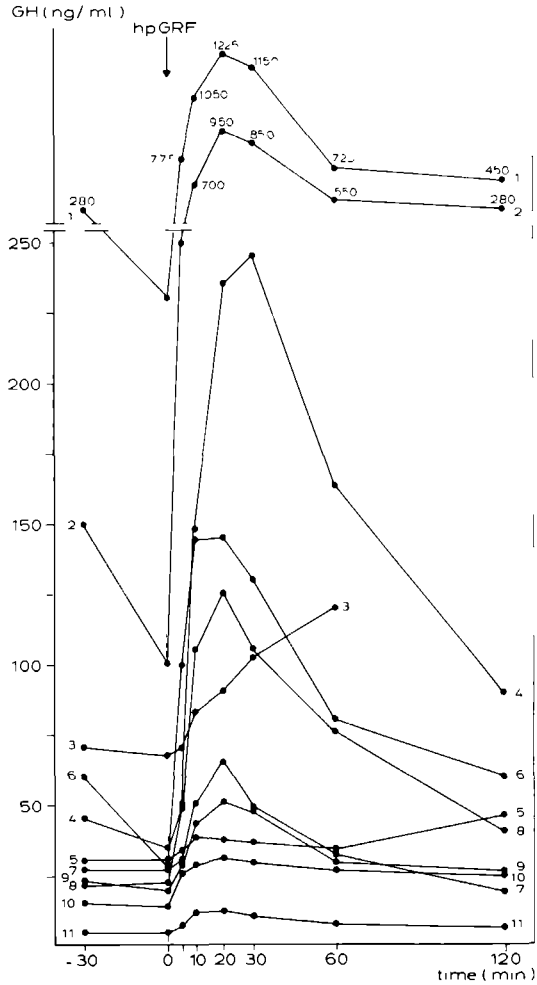


FIG. 1. Individual GH levels before and after hpGRF administration (arrow) in 11 patients with acromegaly. The levels of Patients 1 and 2 are indicated in digits at the top of the figure. Note the tight correlation between basal and stimulated GH levels.

RESULTS

EFFECTS OF hpGRF

Figure 1 illustrates that hpGRF stimulated GH release in all patients with acromegaly. The mean GH level rose from 54 ± 20 ng/ml at 0 min to 215 ± 126 ng/ml after 20 min ($p^{*}<0.001$) and afterwards declined gradually to 103 ± 44 ng/ml after 120 min. In the individual patients, maximal GH values were achieved 20 + 2 min after hpGRF administration with the exception of patient No.3, who showed a rather sluggish response. The relative changes in all patients varied from +57% to +624% of the basal value. It is striking that we found a highly significant positive correlation between the individual GH levels and the maximal GH increases ($p^{**}<0.01$, R_S = +0.80). Thus the higher the GH levels, the greater the response to hpGRF.

EFFECT OF CONCOMITANT SRIF ADMINISTRATION ON hpGRF-INDUCED GH-RESPONSES

Figure 2 illustrates that SRIF, 300 µg/h, lowered GH levels in all patients from a mean value of 76 + 38 ng/ml to 13 ± 5 ng/ml ($p<0.01$) after 1 h. Subsequently hpGRF administration during concomitant SRIF infusion elicited an increase of GH to a mean of 65 + 27 ng/ml after 20 min ($p<0.01$). This response was less than after the administration of hpGRF alone ($p<0.01$). In the individual patients maximal responses during SRIF were obtained 22 + 7 min after hpGRF. The relative changes varied from 40% to 978% (mean 219 + 16%) of the GH levels achieved after 1 hour of SRIF administration. The responses to the combined administration of SRIF and hpGRF amounted to between 4% and 39% (mean 18 ± 5%) of those achieved after hpGRF alone.

The GH levels achieved after 1 h of SRIF-infusion in the individual patients were again positively correlated with the maximal GH increase after hpGRF administration ($p^{**}<0.05$, R_S= +0.79). In the patients whose GH levels were suppressed to levels below 15 ng/ml, the GH responses to hpGRF were almost completely blunted by SRIF with the exception of patient No.2. In the patients with higher GH levels during SRIF, this peptide was unable to block the hpGRF-induced GH response.

DISCUSSION

This study confirms that hpGRF stimulated GH secretion in all 11 patients with acromegaly. The maximal GH response was achieved 10 to 60 min after hpGRF-administration. GH responsiveness appeared to be highly variable

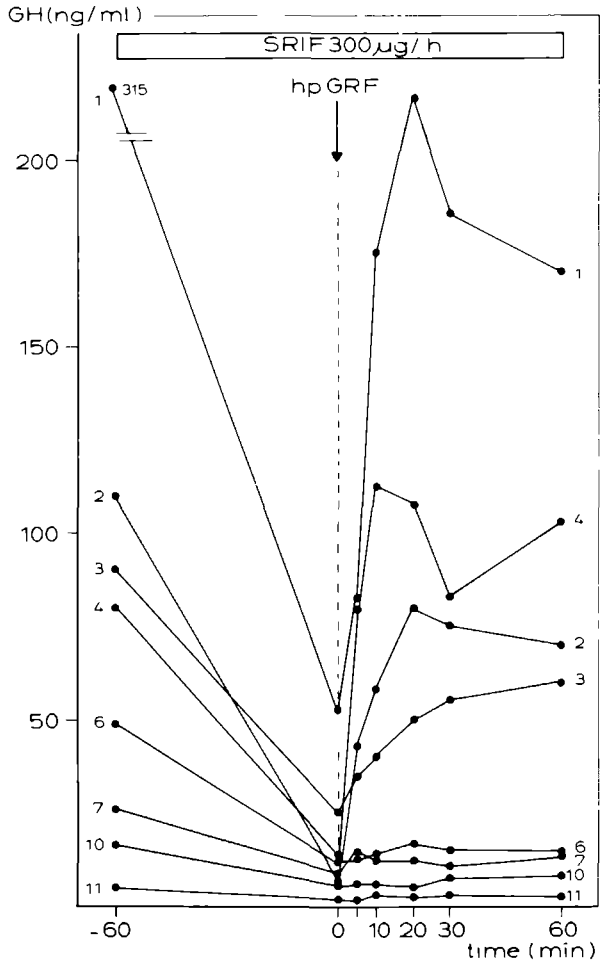


FIG. 2. Individual GH levels before, after one hour of SRIF (300 µq/h) and during concomitant SRIF and hpGRF administration. Arrow marks hpGRF administration and bar marks SRIF.

with responses ranging from 7 to 995 ng/ml. In addition, we found that GH responsiveness to hpGRF was positively correlated with the GH levels, i.e. the higher the GH levels were, the greater was the rise after hpGRF administration. In our opinion this observation argues against hypersecretion of endogenous GRF as the cause of acromegaly in these patients.

GH levels achieved after 1 h of SRIF infusion were positively correlated with the GH values obtained in response to hpGRF during SRIF-blockade, i.e. the lower the GH levels during SRIF administration were, the lower the responsiveness to hpGRF during concomitant SRIF administration.

It is known that SRIF is able to lower GH levels in all patients with acromegaly, though to a highly variable extent as we discussed earlier (Pieters et al.1982). In this study the extent of SRIF-blockade of GH levels in acromegaly appears to concur with the degree of stimulation of the SRIF-blocked pituitaries by hpGRF.

In both circumstances with and without exogenous SRIF, the GH level before hpGRF-administration determines GH responsiveness to hpGRF-administration. A possible explanation for these findings is that in acromegaly the number of GH-producing cells is increased, influencing the GH levels accordingly. Responsiveness to hpGRF is essentially normal and increased pari passu with the increased number of GH-producing cells. Exogenous SRIF is able to inhibit GH release and GH synthesis. The more the GH levels decline during SRIF administration, the smaller the intracellular stores of GH are expected to be causing a comparable decrease of GH responsiveness to hpGRF.

Our in vivo data differ from the in vitro data obtained using pituitary tissue from patients with acromegaly as mentioned above. The difference between the in vitro experiments with SRIF and hpGRF and our in vivo data might be explained by: (i) the great variability in SRIF sensitivity between acromegalic patients; (ii) differences in the doses of SRIF used in the two types of experiments; and (iii) differing behaviour of the pituitary tissue when removed from its natural environment.

Finally, our data do not favour the concept of autonomously GH producing cells in acromegaly, nor a hypothalamic cause of this disease, but rather argue for principally normal responsiveness to both SRIF and hpGRF at a higher setting of basal GH levels. It is therefore likely that long-acting SRIF analogues will be of benefit in the treatment of acromegaly.

REFERENCES

Adams, E., Winslow, C. & Mashiter, K. (1983a) Pancreatic growth hormone releasing factor stimulates growth hormone secretion by pituitary cells. Lancet, i, 1100-1101.

Adams, E., Winslow, C. & Mashiter, K. (1983b) In vitro effects of hpGRGF 1-40 and hpGRF 1-44 on growth hormone secretion from human somatotrophic tumours. Acta Endocrinologica (Copenh), 103, (suppl. 256, abstr. 126), 70.

Brazeau, P., Ling, N., Bohlen, P., Esch, F., Ying, S. & Guillemin, R. (1983) Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone in vitro. Proceedings of the National Academy of Sciences of the United States of America 79, 7909-7913.

Di Chiro, G. & Nelson, K. (1962) The volume of the sella turcica. American Journal of Roentgenology, 87, 989-994.

Gelato, M., Pescovitz, O., Cassorla, F., Loriaux, D. & Merriam, G. (1983a) Effects of a growth hormone releasing factor in man. Journal of Clinical Endocrinology and Metabolism, 57, 674-676.

Gelato, M., Rock, J., Borges, J., Oldfield, E., Vale, W., Loriaux, D., Thorner, M. & Merriam, G. (1983b) Growth hormone releasing factor stimulation of growth hormone in acromegaly. Acta Endocrinologica (Copenh), 113, (suppl 256, abstr.128), 71.

Guillemin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N. & Wehrenberg, W.B. (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science, 218, 585-587.

Harwood, J. & Grewe, C. (1983) Actions of growth hormone releasing factor (GRF) and somatostatin 8SRIF) on growth hormone and cyclic AMP production in cultured anterior pituitary cells. 65th Annual Meeting of the Endocrine Society, San Antonio, p 83, Abstr 10.

Lamberts, S., Verleun, T. & Oosterom, R. (1984) The interrelationship between the effects of somatostatin and human pancreatic growth hormone releasing factor on growth hormone release by cultured pituitary tumor cells from patients with acromegaly. Journal of Clinical Endocrinology and Metabolism, 58, 250-254.

Pieters, G., Romeyn, J., Smals, A. & Kloppenborg, P. (1982) Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. Journal of Clinical Endocrinology and Metabolism, 54, 942-948.

Rivier, J., Spiess, J., Thorner, M. & Vale, W. (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature, 300, 276-278.

Rosenthal, S., Schriock, C., Kaplan, S., Guillemin, R. & Grumbach, M. (1983) Synthetic human pancreatic growth hormone releasing factor stimulates GH secretion in normal men. Journal of Clinical Endocrinology and Metabolism, 57, 677-679.

Shibasaki, T., Shizume, K., Masuda, A., Nakahara, M., Hizuka, M., Miyakawa, M., Takano, K., Demura, H., Wakabayashi, I. & Ling, N. (1984) Plasma growth hormone response to growth hormone-releasing factor in acromegalic patients. Journal of Clinical Endocrinology and Metabolism, 58, 215-217.

Smals, A., Kloppenborg, P., van Haelst, U., Lequin R. & Benraad, Th. (1978) Fertile eunuch syndrome versus classic hypogonadism. Acta Endocrinologica (Copenh), 87, 389-395.

Thorner, M., Spiess, J., Vance, M., Rogol, A., Kaiser, D., Webster, J., Rivier, J., Borges, J., Bloom, S., Cronin, M., Evans, W. & MacLeod, R. (1983) Human pancreatic growth-hormone-releasing-factor selectively stimulates growth-hormone secretion in man. Lancet, i, 24-28.

Vale, W., Vaughan, J., Yamamoto, G., Spiess, J. & Rivier, J. (1983) Effects of synthetic human pancreatic (tumor) GH releasing factor and

somatostatin, triiodothyronine and dexamethasone on GH secretion in vitro. Journal of Clinical Endocrinology and Metabolism, 112, 1553-1555.

von Werder, K., Muller, O., Buchner, A., Hartl, R., Losa, M. & Stalla, G. (1983) Human pancreatic growth hormone releasing factor (hpGRF)· Biological activity in normal controls. Acta Endocrinologica (Copenh), 103, suppl. 256, abstr. 127), 71

Webb, C., Thominet, J. & Frohman, L. (1983) Ectopic growth hormone releasing factor stimulates growth hormone release from human somatotroph adenomas in vitro. Journal of Clinical Endocrinology and Metabolism, 57, 417-419.

Wehrenberg, W., Ling, N., Bohlen, P., Esch, P., Esch, F., Brazeau, P. & Guillemin, R. (1982a) Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. Biochemical and Biophysical Research Communications, 109, 562-567.

Wehrenberg, W., Ling, N., Brazeau, P., Esch, F., Bohlen, P., Baird, A., Ying, S. & Guillemin, R. (1982b) Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. Biochemical and Biophysical Research Communications, 109, 382-387.

Wood, S., Ch'ng, J., Adams, E., Webster, J., Joplin, G., Mashiter, K. & Bloom, S. (1983) Abnormalities of growth hormone release in response to human pancreatic growth hormone releasing factor (GRF 1-44) in acromegaly and hypopituitarism. British Medical Journal, 286, 1687-1691.

Chapter 4.5

THE EFFECT OF MINISOMATOSTATIN ON ANOMALOUS GROWTH HORMONE RESPONSES IN ACROMEGALY

GFFM Pieters, AEM Smals, AGH Smals, JA v Gennep[1] and PWC Kloppenborg

Division of Endocrinology, Department of Medicine, Radboud Hospital, University of Nijmegen, Nijmegen, and Sandoz B.V. Netherlands[1], Loopkantstraat, Uden, The Netherlands

ABSTRACT

Twelve patients with active acromegaly were treated with the long-acting somatostatin analogue SMS 201-995 at a dose of 50 μg sc twice daily in the first 2 weeks of treatment and 100 μg twice daily thereafter. Four hours after the first injection of SMS, GH levels became normal in 8 of the 12 patients. The GH response after GHRH administration was strongly suppressed by SMS. Paradoxical GH responses to TRH disappeared in 6 out of 7 patients during SMS. Paradoxical responses to LHRH, however, persisted in 4 out of 4 patients. Paradoxical responses of GH after glucose loading disappeared in 2 out of 2 patients. We conclude that SMS normalizes most anomalous growth hormone kinetics in acromegaly. This drug offers a new tool in the treatment of this disease.

Acta Endocrinol (Copenh) 1987 (114): 537-542

INTRODUCTION

In acromegaly, paradoxical increases in GH levels occur in about 25% of the patients after oral glucose loading (Beck et al.1966), in approximately 60% after the administration of thyrotropin-releasing hormone (TRH) (Irie & Tshushima 1972), and in about 25% after luteinizing hormone releasing hormone (LRH) (Rubin et al.1973; Pieters et al.1982). The response of the GH levels to growth hormone releasing hormone (GHRH) administration is generally enhanced in acromegaly (Wood et al.1983; Pieters et al.1984).

Patients whose GH levels rise after TRH administration are more sensitive to the GH inhibitory effect of dopaminergic drugs (Liuzzi et al. 1974) whereas those whose GH levels rise after LRH are more prone to the inhibitory effect of somatotropin-releasing inhibiting factor (SRIF) (Pieters et al.1982). During bromocriptine treatment, the paradoxical GH response to LRH disappears, but not the response to TRH (Ishibashi et al.1977,1978). The GH response after GHRH administration also persists during bromocriptine treatment (Cozzi et al.1986).

Until now, only few data are available on the ability of SRIF to blunt the anomalous GH responses to the stimuli mentioned above. With the development of the long-acting somatostatin analogue SMS 201-995 (minisomatostatin, SMS) it became possible to normalize the GH levels in the majority of patients with acromegaly (Bauer et al.1982; von Werder et al.1984; Plewe et al.1984; Ch'ng et al.1985; Althoff et al.1984; Lamberts et al.1985,1986; Pieters et al.1986). We studied the effects of 4 weeks of treatment with SMS on the GH responses to glucose loading, TRH, LRH and GHRH administration in 12 patients with acromegaly.

MATERIALS AND METHODS

Twelve patients with active acromegaly, 9 women (mean age ± SE, 50 ± 5 years) and 3 men (39 ± 7 years), participated in this study. Informed consent was obtained from all patients after approval of the protocol by the hospital ethical committee. All patients were resistant to bromocriptine therapy or developed serious adverse reactions.

TRH (200 μg, Roche Ltd, Basle, Switzerland), LRH (100 μg, Hoechst A.G., Frankfurt a.M., FRG), and GHRH ($hpGRF_{1-44}$, 100 μg, Bachem Ltd, Torrance CA) were administered in random order on separate days at 09.00 h with the patients fasting and at bed rest. Blood samples for hormone assays were obtained via an indwelling iv cannula at -30, 0, 5, 10, 20, 30, 60 and 120 min after the administration of the releasing hormone. On a separate occasion during the study period, an oral glucose tolerance test (100 g of glucose) was also performed at 09.00 h. In the fifth week of SMS

treatment, the glucose tolerance test and the GHRH test were repeated and so were those releasing hormone tests to which a paradoxical response occurred - according to the criteria reported earlier, i.e. an increase in GH levels exceeding 50% above baseline in at least 2 samples and within 30 min (Pieters et al.1982). SMS was provided by Sandoz A.G., Basle, Switzerland, and was administered sc at a dose of 50 μg twice a day at 08.00 and 20.00 h. After 2 weeks of treatment, the dose was increased to 100 μg twice daily. Blood samples for hormone assay were obtained hourly from 08.00 to 12.00 h the day before SMS treatment, the first day of treatment, and in the fifth week of treatment.

The growth hormone levels were determined by a specific radio-immunoassay as described before: GH (intra-assay coefficient of variation, CV 15%) (Pieters et al.1982).

RESULTS

BASAL GH LEVELS BEFORE AND DURING SMS ADMINISTRATION

On the first day of treatment, the plasma GH levels declined in all patients from a mean basal value of 73 ± 20 μg/l to a nadir value of 10 ± 4 μg/l after 3-4 h (Fig.1). The GH levels normalized (< 7.5 μg/l) in 8 of the 12 patients. This GH lowering effect of SMS persisted during the following weeks of treatment (data not shown).

GHRH ADMINISTRATION

All patients who underwent a GHRH test before and during SMS treatment, except Nos. 6 and 8 who had the lowest basal GH levels, showed fair GH responses after GHRH administration (Fig.2). After SMS administration, GH responsiveness to GHRH completely disappeared in 3 of the 8 responders and was almost completely blunted in 4. The responsiveness to GHRH persisted in only one patient (No.5), although the maximal GH response was 35% lower than before treatment.

TRH ADMINISTRATION

Seven patients showed paradoxical responsiveness of GH levels to TRH administration before SMS treatment (Fig.3). During SMS, this paradoxical responsiveness completely disappeared except in patient No.6. In this patient, the paradoxical responsiveness persisted, although the maximal increase was only 30% of the response before treatment.

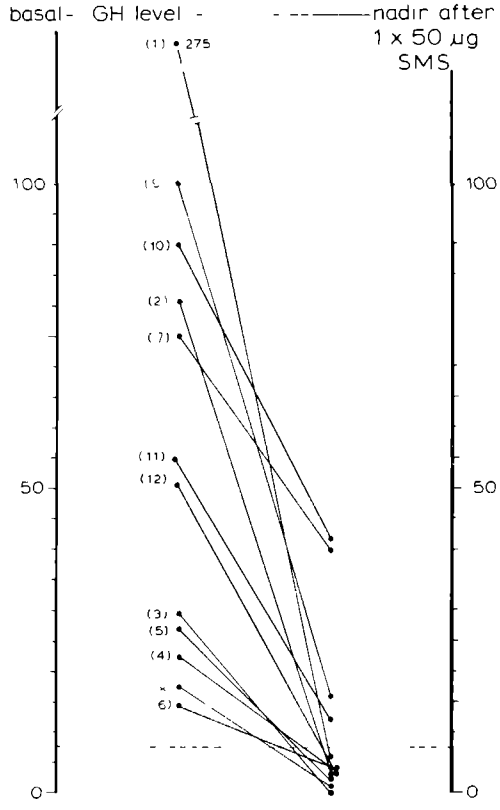


FIG. 1. Individual basal GH levels (µg/l) and GH levels (µg/l) 4 h after the first injection of SMS (50 µg sc) in 12 patients with acromegaly. The figures in parentheses indicate the individual patients.

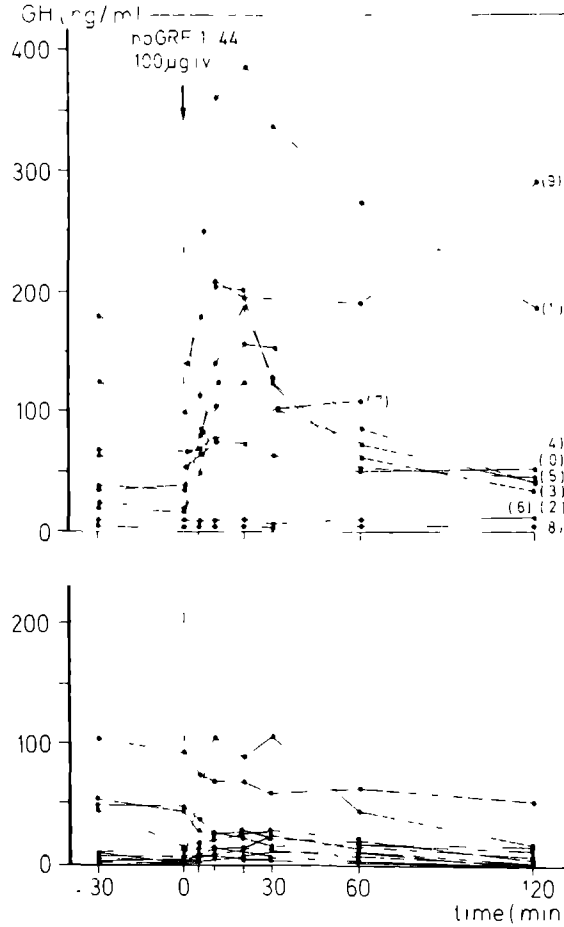


FIG. 2. Individual GH responses after GHRH administration before (upper panel) and during treatment with SMS, 100 µg sc twice daily (lower panel).

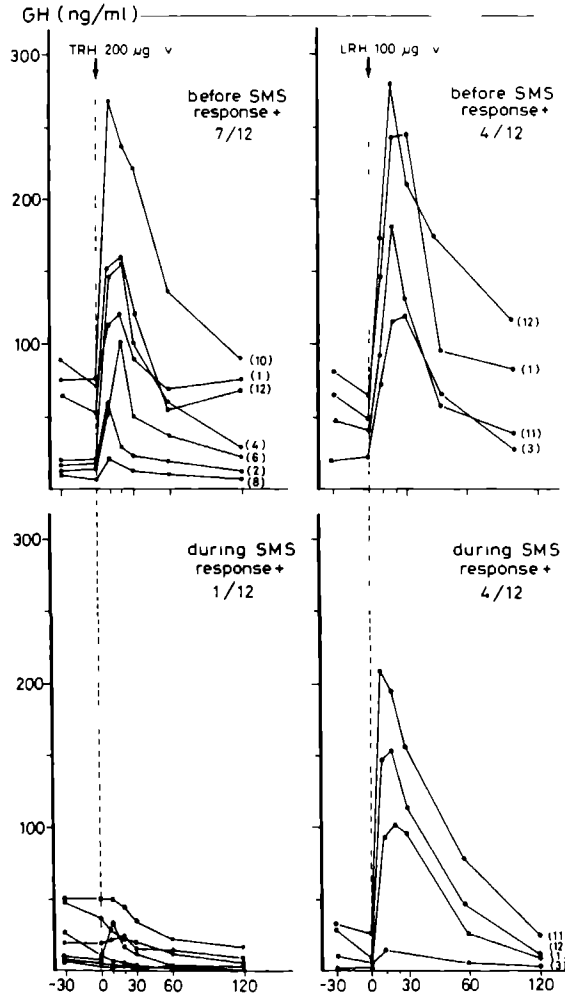


FIG. 3. Individual GH responses of paradoxically responding patients to TRH (left panel) and LHRH (right panel) before SMS (upper panel) and during SMS, 100 µg twice daily (lower panel).

LRH ADMINISTRATION

Before treatment, 4 patients responded paradoxically to LRH. In all these patients, paradoxical responsiveness persisted during SMS with a mean maximal GH increase of 70% in comparison to the response before treatment.

GLUCOSE ADMINISTRATION

GH levels showed a paradoxical increase during glucose loading in 2 patients out of 8 nondiabetic acromegalics. This paradoxical response disappeared during SMS administration in both patients.

DISCUSSION

The results in this study on the acute effects of SMS 201-995 on GH secretion in acromegaly are in agreement with those of other investigators (Bauer et al.1982; von Werder et al.1984; Plewe et al.1984; Ch'ng et al.1985; Althoff et al.1984; Lamberts et al.1985,1986; Pieters et al.1986) who reported normalization of GH in the majority of the patients after injection of 50 μg SMS.

The GH responses after GHRH admnistration are exaggerated in patients with acromegaly with high basal GH levels (Pieters et al.1984). Earlier we described that native somatostatin (SRIF) given as a continuous iv infusion at a dose of 300 μg per hour was not able to blunt completely the GH responses after GHRH injection (Pieters et al.1984). In the present study we observed that GHRH responsiveness disappears almost completely during chronic SMS administration. This discrepancy between the effects of SRIF and SMS may be due to differences in efficacy of the dosis used in the acute SRIF test and the chronic SMS treatment.

The TRH-induced paradoxical growth hormone release was completely blunted in all patients except one. These data are at variance with those of other investigators who failed to demonstrate an effect of SRIF, 100 μg per hour iv, on TRH-induced GH release in acromegalics (Giustina et al.1974; Faglia et al.1975). It was later demonstrated that the paradoxical GH response to TRH was blunted during the infusion of 1000 μg of SRIF per hour (Gomez-Pan et al.1975). Again differences in the dose of somatostatin may account for these seemingly contradictory results.

Interestingly, SMS could not prevent the paradoxical responsiveness of GH after LRH administration in any of the 4 patients who showed such a response. Similar results on LRH-responsiveness during SRIF infusion have been reported earlier (Giustina et al.1974), although these authors used a

rather inappropriate dose of 100 µg of SRIF per hour.

It has been suggested that the anomalous GH responses after TRH and/or LRH administration occur by dedifferentiation of the GHRH-receptor of the somatotrophs in the adenomas (Liuzzi et al.1974). According to this dedifferentiation, TRH and/or LRH should stimulate GH release via a mechanism similar to that of GHRH. If this hypothesis were true, one would expect that the GH responses to TRH and LRH would disappear during an SMS administration that is comparable to the blunting of the GH responses after GHRH administration.

Our data and those of other investigators illustrate the disparity of the effects of SMS and bromocriptine on the dynamics of GH secretion in patients with acromegaly. Chronic treatment with SMS blunts the response of the somatotrophs to TRH and to GHRH completely in the great majority of the patients, but leaves the response to LRH, if present, largely unaffected. In turn chronic treatment with bromocriptine leaves the response to GHRH intact (Cozzi et al.1986) and reduces the response to TRH only by approximately 50% (Ishibashi et al.1977; Cozzi et al.1986), but it completely blunts the response to LRH, if present (Ishibashi et al.1978).

Thus, the effects of SMS and bromocriptine on the dynamics of GH secretion in acromegaly to a certain extent are complementary.

From these data we infer that combined treatment with SMS and bromocriptine may be beneficial in some patients with acromegaly who do not successfully respond to treatment with SMS or bromocriptine alone, as was demonstrated recently (Lamberts et al.1986).

REFERENCES

Althoff PH, Boetger B, Rosak C, Neufeld M, Kacey F, Knigge H, Magnet W, Jungmann E & Schöffling K (1984): Influence of a long acting somatostatin analogue (SMS 201-995) in acromegalic patients. Acta Endocrinol (Copenh). Suppl 264: 29. Abstr 32.

Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P & Petcher TJ (1982): SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci 31: 1133-1135.

Beck P, Parker ML & Daughaday WH (1966): Paradoxical hypersecretion of growth hormone in response to glucose. J Clin Endocrinol Metab 26: 243-248.

Ch'ng LJC, Sandler LM, Kraenzlin ME, Burrin JM, Joplin GF & Bloom SR (1985): Long term treatment of acromegaly with a long acting analogue of somatostatin. Br Med J 290: 284-285.

Cozzi R, Dallabonzona D, Oppizzi G, Verde G, Liuzzi A & Chiodini P (1986): Bromocriptine does not alter growth hormone GH) responsiveness to GH releasing hormone in acromegaly. J Clin Endocrinol Metab 62: 601-604.

Faglia G, Paracchi A, Beck-Peccoz P & Ferrari C (1975): An explanatory hypothesis for plasma GH response to non-specific releasing hormones and for paradoxical GH inhibition after dopaminergic drugs in acromegaly.

Acta Endocrinol (Copenh). Suppl 199: 323. Abstr 253.
Giustina G, Reschini E, Perachi M, Cantalamessa L, Cavaginini F, Pinto M & Bulgheroni P (1974): Failure of somatostatin to suppress thyrotropin releasing factor and luteinizinig hormone releasing factor induced growth hormone release in acromegaly. J Clin Endcrinol Metab 38: 906-909.
Gomez-Pan A, Tunbridge WMG, Duns A, Hall R, Besser GM, Coy DH, Schally AV & Kastin AJ (1975): Hypothalamic hormone interaction in acromegaly. Clin Endocrinol (Oxf) 4: 455-460.
Irie M & Tsushima T (1972): Increase of serum growth hormone concentration following thyrotropin releasing hormone injection in patients with acromegaly or gigantism. J Clin Endocrinol Metab 35: 97-100.
Ishibashi M, Yamaji T & Kosaka K (1977): Effect of bromocriptine on TRH induced growth hormone and prolactin release in acromegalic patients. J Clin Endocrinol Metab 45: 275-279.
Ishibashi M, Yamaji T & Kosaka K (1978): Induction of growth hormone and prolactin secretion by luteinizing hormone-releasing hormone and its blockade by bromoergocriptine in acromegalic patients. J Clin Endocrinol Metab 47: 418-421.
Lamberts SWJ, Uitterlinden P, Verschoor L, van Dongen KJ & del Pozo E (1985): Long term treatment of acromegaly with the somatostatin analogue SMS 201-995. N Engl J Med 313: 1576-1580.
Lamberts SWJ, Zweens M, Verschoor L & del Pozo E (1986): A comparison among the growth hormone lowering effects in acromegaly of the somatostatin analogue SMS 201-995, bromocriptine, and the combination of both drugs. J Clin Endocrinol Metab 63: 16-19.
Liuzzi A, Chiodini PG, Botalla L, Silvestrini F & Müller EE (1974): Growth hormone (GH) releasing activity of TRH and GH lowering effect of dopaminergic drugs in acromegaly: homogeneity in the two responses. J Clin Endocrinol Metab 39: 871-876.
Pieters GFFM, Romeijn JE, Smals AGH & Kloppenbog PWC (1982): Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. J Clin Endocrinol Metab 54: 942-948.
Pieters GFFM, Smals AEM, Hermus ARMM, Smals AGH, Benraad ThJ & Kloppenborg PWC (1984): Growth hormone responsiveness to human pancreatic growth hormone releasing factor in acromegaly: modulatory effects of basal hormone levels and of concomitant somatostatin administration. Clin Endocrinol (Oxf) 21: 701-707.
Pieters GFFM, Smals AGH & Kloppenborg PWC (1986): SMS 201-995 in acromegaly. N Engl J Med 314: 1390-1392.
Plewe G, Krause U, Beyer J, Neufeld M & del Pozo E (1984): Long-acting and selective suppression of growth hormone by somatostatin analogue SMS 201-995 in acromegaly. Lancet 2: 782-784.
Rubin AL, Levin SR, Burnstein RI, Tyrell B, Biacci C & Forsham PH (1973): Stimulation of growth hormone by luteinizing hormone releasing hormone in active acromegaly. J Clin Endocrinol Metab 27: 160-162.
von Werder K, Losa M, Müller DA, Schweiberer L, Fahlbusch R & del Pozo E (1984): Treatment of metastasising GRF-producing tumour with a long acting somatostatin analogue. Lancet 2: 282-283.
Wood S, Ch'ng LJC, Adams E, Webster J, Joplin G, Mashiter K & Bloom S (1983): Abnormalities of growth hormone release in response to human pancreatic growth hormone releasing factor (GRF 1-44) in acromegaly and hypopituitarism. Br Med J 286: 1687-1691.

Chapter 5

COMMENTS

An intriguing observation in this thesis is the finding of a sexual dimorphism in GH responsiveness to GHRH between young adult men and women in favour of the men, whereas in midpubertal (tall) adolescents the reverse is true i.e. a twice higher GH response to GHRH in the girls as compared to the boys. The finding of a more pronounced GH response to GHRH in the pubertal girls is reminiscent of earlier studies, reporting a sex difference in GH release after arginine stimulation (1). It is also in line with data of Gelato et al.(2) using GHRH testing in normal statured midpubertal adolescents. These data all point to a more intensive hypothalamic drive in GH secretion from the somatotroph in midpubertal girls than in boys, which is also reflected by the presence of higher circulating GHRH levels in the former (3). The data in human adolescents fit with those obtained in pubertal rats, which show a similar sex difference in GH responsiveness to GHRH in favour of the female, both in vivo and in vitro (4). There is much more controversy with respect to the sex difference in GH response to GHRH in young adults. In 22 carefully selected healthy adults in their early twenties with normal Rohrer-indices an overt sex difference in GH responsiveness to GHRH was found, the GH increase in men being almost twice that in women. These findings contrast, however, with data from other studies, which either reported no sex difference in GH responsiveness between adult men and women (5,6) or a somewhat higher response in women (7,8). Lang et al.(7) reported higher GH peak increments in premenopausal women (< 30 to 50 yr) than in age matched males. The maximum GH responses to GHRH and the area under the GH curve were positively correlated with plasma oestradiol levels. No sex difference in GH response was found in older subjects. In a recent report the group of Besser (9) reported an overt effect of oestrogen administration on the GH response to insulin-induced hypoglycemia, but not to GHRH in short statured girls. Evans et al.(10) and Barbarino et al.(8) did not find any difference in GH responsiveness to GHRH during the menstrual cycle, which argues against a major role of oestrogens in modulating the GH response. We have no explanation for the discrepancy between our data and those of Lang et al.(7) and Barbarino et al.(8), although they partially may be due to the large interindividual variation in GH responsiveness to GHRH. Nevertheless, our data are in line with those in animal studies. In intact adult male rats the GH response to GHRH is more pronounced than in intact females (11). The male pituitary contains a significantly greater percentage of somatotrophs, the GH secretion in vitro is higher in response to GHRH whereas the sensitivity of the male somatotroph is about 5 times that of females

(11,12). Testosterone treatment of castrated male rats, but not oestradiol, stimulates the GH response to GHRH (11). Recently Ohlson et al.(13) also demonstrated that testicular androgen secretion in adult male rats increases pituitary GH release in response to GHRH in vitro, whereas ovarian oestrogen secretion is of less importance for the GHRH responsiveness of female rat pituitaries. In human adults not only a sex difference in GH responsiveness to GHRH is present but also sexual dimorphism in the endogenous opioidergic modulation of pituitary GH secretion. Administration of the opiate receptor antagonist nalaxone was capable of inhibiting GH release induced by direct stimulation with GHRH in young women, but not in age matched male controls (8). The absence of such an effect in normal men strongly indicates a sex dependence of naloxone effects and suggests a role of the sex steroids in modulating pituitary GH secretion (8). Further studies are needed to precisely define the sex difference in GH responsiveness to GHRH between men and women in different age groups and to assess the role of sex steroids in this response.

Besides these observations on some aspects of the physiology of GH-GHRH relations, the second part of this thesis, mainly deals with studies in patients with pathological hypersecretion of growth hormone. Strong additional evidence was obtained for the hypothesis that in GH secreting adenomas, cells sensitive to GHRH and less to TRH and Br (pure somatotrophs) may coexist with cells responsive to TRH and Br but less to GHRH (lactotroph-like cells) (14, Chapter 4.2). Remarkably we observed such relations only in patients who showed a paradoxical GH response to TRH, not in the TRH "non-responders" (Chapter 4.3). Only in the TRH responders the GH response to Br was linked to the basal prolactin level, whereas also the prolactin increments to GHRH and TRH were closely related, suggesting according to Chiodini et al.(14) dedifferentiation of the somatotrophs in the adenoma to a more primitive lactotroph-like (GH producing) cell. Indeed, GH suppression by Br appeared more pronounced in the acromegalic patients responding to TRH than in the non-responders. Nonetheless, also in these latter patients with allegedly more pure somatotroph adenomas, Br reduced GH levels to less than 50% of the preteatment value. These apparently confusing data became more intelligible in the light of a very recent publication of Koga et al.(15), demonstrating the presence of specific dopamine receptors even in 8 out of 14 pure GH secreting adenomas.

Remarkable is also the close relation between GH responsiveness to GHRH and LHRH and between the responses to LHRH and SRIF at least in TRH non-responsive acromegalics (Chapter 4.3). Recently Faglia et al.(16) noted that the higher GH responses to GHRH were found in patients paradoxically responding to LHRH. Earlier Pieters et al.(17) demonstrated that paradoxi-

cal GH responses to LHRH mainly occurred in acromegalic patients, with a high sensitivity to somatostatin. In view of these close relations it was expected that GH suppression by SRIF would be more pronounced in the patients with allegedly pure GH producing adenomas (TRH non-responders) than in those with mixed, lactotroph-like adenomas (TRH-responders). Surprisingly, however, GH suppression by somatostatin appeared similar in both groups of acromegalics. Lamberts et al.(18) administering the long acting somatostatin analogue MS 201-995 reported favourable GH responses to the drug in 3 out of 5 acromegalic patients with pure GH secreting adenomas and in 3 out of 4 with mixed GH/Prl adenomas. An additive response of SMS and Br was observed in 2 out of 3 patients with the pure adenoma and in all acromegalics with the mixed adenoma. The in vivo data paralleled the findings in vitro (19,20). In a recent study Moyse et al.(21) and Reubi et al.(22) using autoradiographical and/or immunohistochemical staining, demonstrated the presence of specific somatostatin receptors in about 70% of pure GH secreting adenomas as well as in mixed GH/Prl tumors. Together with the data on the presence of dopamine receptors in a subset of pure GH adenomas and in almost all mixed tumors (15), the presence of SRIF receptors in both types of tumor in most, but not all acromegalic patients, may explain the clinically variable response to each of the drugs or to their combined administration. Testing the sensitivity to Br and/or SRIF in acromegalic patients in vivo or in vitro may therefore be worth to determine the treatment of choice in acromegaly. Testing GH responsiveness to GHRH and also to TRH and LHRH, although interesting for a better understanding of the pathogenesis of acromegaly, hardly has therapeutical implications. Dynamic testing of GH secretion by means of GHRH, TRH or LHRH is, however, of value to assess the residual GH secretion of the somatotroph during treatment with SMS or Br. The GH responses to GHRH and to TRH are dramatically blunted or even disappear completely in most acromegalic patients treated with SMS (23,24, Chapter 4.5), whereas the paradoxical GH response to LHRH persists (Chapter 4.5). In turn chronic treatment with Br leaves the response to GHRH intact (25), reduces the answer to TRH by approximately 50%, but completely blunts the paradoxical answer to LHRH (26). These findings are rather unexpected in the light of the earlier described tight associations between the GH responses to GHRH, somatostatin and LHRH in a subset of acromegalics and the inverse relation between GH responsiveness to GHRH at one end and to TRH and Br at the other in most patients. It has to be emphasized, however, that these associations were found during acute testing using different doses of Br and short acting native somatostatin. Nevertheless the data again suggest that the effects of SMS and Br on GH secretion are mediated via separate pathways, involving different receptors as illustrated earlier. The complementary effects of Br and the more potent long acting

somatostatin analogue (24,27) on GH secretion in acromegalics, justify combined treatment in patients who only partially respond to one of either drugs.

REFERENCES

1. Sperling MA, Kenny FM, Drash AL (1970) Arginine-induced growth hormone responses in children: effect of age and puberty. J Pediatr 77: 462.
2. Gelato MC, Malozowski S, Caruso-Nicoletti M (1986) Growth hormone (GH) responses to GH-releasing hormone during pubertal development in normal boys and girls: comparison to idiopathic short stature and GH-deficiency. J Clin Endocrinol Metab 63: 174.
3. Argente J, Evain-Brion D, Munoz-Villa A, Garnier P, Hernandez M, Donnadieu M (1986) Relationship of plasma growth hormone-releasing hormone levels to pubertal changes. J Clin Endocrinol Metab 63: 680.
4. Hertz P, Silbermann M, Youdim MBH, Hochberg Z (1987) Sexual dimorphism of growth hormone (GH) secretion by cultured pituicytes of young gonadectomized rats. First European Congress of Endocrinology (Copenhagen) Abstr 21-595: 156.
5. Gelato MC, Pescovitz OH, Cassorla F, Loriaux DL, Merriam GR (1984) Dose-response relationships for the effects of growth hormone-releasing factor-(1-44)-NH_2 in young adult men and women. J Clin Endocrinol Metab 59: 197.
6. Sheldon WR Jr, DeBold CR, Evans WS, Decherney GS, Jackson RV, Island DP, Thorner MO, Orth DN (1985) Rapid sequential intravenous administration of four hypothalamic releasing hormones as a combined anterior pituitary function test in normal subjects. J Clin Endocrinol Metab 60: 623.
7. Lang I, Schernthaner G, Pietschmann P, Kurz R, Stephenson JM (1987) Growth hormone response to growth hormone-releasing hormone is independently affected by both sex and age in healthy individuals. First European Congress of Endocrinology (Copenhagen) Abstr 1-11: 5.
8. Barbarino A, de Marinis L, Mancini A, d'Amico C, Passeri M, Zuppi P, Sambo P, Tofani A (1987) Sex-related naloxone influence on growth hormone-releasing hormone-induced growth hormone secretion in normal subjects. Metab 36: 105.
9. Ross RJM, Grossman A, Davies PSW, Savage MO, Besser GM (1987) Stilboestrol pretreatment of children with short stature does not affect the growth hormone response to growth hormone-releasing hormone. Clin Endocrinol 27: 155.
10. Evans WS, Borges JL, Vance ML, Kaiser DL, Rogol AD, Furlanetto R, Rivier R, Vale W, Thorner MO (1984) Effects of human pancreatic growth hormone-releasing factor-40 on serum growth hormone, prolactine, luteinizing hormone, follicle-stimulating hormone, and somatomedin-C concentrations in normal woman throughout the menstrual cycle. J Clin Endocrinol Metab 59: 1006.
11. Evans WS, Krieg RJ, Limber ER, Kaiser DL, Thorner MO (1985) Effects of in vivo gonadal hormone environment on in vitro hGRF-40-stimulated GH release. Am J Physiol 249: E 276.
12. Frohman LA, Jansson J (1986) Growth hormone-releasing hormone. Endocrine Reviews 7: 223.

13. Ohlsson L, Isaksson O, Jansson JO (1987) Endogenous testosterone enhances growth hormone (GH)-releasing factor-induced GH secretion in vitro. J Endocrinol 113: 249.
14. Chiodini PG, Liuzzi A, Dallabonzana D, Oppizzi G, Verde GG (1985) Changes in growth hormone (GH) secretion induced by human pancreatic GH releasing hormone-44 in acromegaly: a comparison with thyrotropin releasing hormone and bromocriptine. J Clin Endocrinol Metab 60: 48.
15. Koga M, Nakao H, Arao M, Sato B, Noma K, Morimoto J, Kishimoto S, Mori S, Kozami S (1987) Demonstration of specific dopamine receptos on human pituitary adenomas. Acta Endocrinol (Copenh) 114: 595.
16. Faglia G, Arosio M, Guglielmino L, Ambrosi B, Sartorio A, Beck-Peccoz P, Spada A, Bochicchio D (1987) Testing acromegaly, In Growth hormone, growth factors and acromegaly (Editors Ludecke DK, Tolis G.) Raven Press, New York, page 135.
17. Pieters GFFM, Romeijn JE, Smals AGH, Kloppenborg PWC (1982) Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. J Clin Endocrinol Metab 54: 942.
18. Lamberts SWJ, Zweers M, Klijn JGM, Vroonhoven van CCJ, Stefanko SZ, Del Pozo E (1986) The sensitivity of growth hormone and prolactin secretion to the somatostatin analogue SMS 201-995 in patients with prolactinomas and acromegaly. Clin Endocrinol 25: 201.
19. Lamberts SWJ, Verleun T, Hofland L, Del Pozo E (1987) A comparison between the effects of SMS 201-995, bromocriptine and a combination of both drugs on hormone release by the cultured pituitary tumour cells of acromegalic patients. Clin Endocrinol 27: 11.
20. Kelijman M, Williams TC, Downs TR, Frohman LA (1987) Comparison of the sensitivity of growth hormone (GH) secretion to somatostatin (SRIF) in vivo and in vitro in acromegaly. Clin Research 35: 585A.
21. Moyse E, Le Dafnier M, Epelbaum J, Pagesy P, Peillon F, Kordon C, Enjalbert A (1985) Somatostatin receptors in human growth hormone and prolactin-secreting pituitary adenomas. J Clin Endocrinol Metab 61: 98.
22. Reubi JC, Heitz PU, Landolt AM (1987) Visualisation of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: evidence for different tumor subclasses. J Clin Endocrinol Metab 65: 65.
23. Barnard LB, Lamberton P, Jackson IMD (1987) Somatostatin analogue (SMS 201-995) in acromegaly: effect on dynamic tests of growth hormone release. Clin Research 35: 582A.
24. Chiodini PG, Cozzi R, Dallabonzana D, Oppizzi G, Verde G, Perroncini M, Liuzzi A, Del Pozo E (1987) Medical treatment of acromegaly with SMS 201-995, a somatostatin analog: a comparison with bromocriptine. J Clin Endocrinol Metab 64: 447.
25. Cozzi R, Dallabonzana D, Oppizzi G, Verde G, Iiuzzi A, Chiodini PG (1986) Bromocriptine does not alter growth hormone (GH) responsiveness to GH releasing hormone in acromegaly. J Clin Endocrinol Metab 62: 601.
26. Ishibashi M, Yamaji, Kosaka K (1978) Induction of growth hormone and prolactin secretion by luteinizing hormone-releasing hormone and its blockade by bromoergocriptine in acromegalic patients. J Clin Endocrinol Metab 47: 418.
27. Liuzzi A, Chiodini PG, Cozzi R, Dallabonzana D, Oppizzi G, Verde G (1987) Hypothalamic releasing and inhibiting hormones in the control

of GH secretion in acromegaly. First European Congress of Endocrinology (Copenh), Abstr 1-4: 3.

SAMENVATTING

In dit proefschrift worden de effecten beschreven van intraveneuze (i.v.) toediening van groeihormoon-"releasing" hormoon (GHRH) aan gezonde jonge volwassenen, aan te lange adolescenten, patiënten met het syndroom van Cushing en in het bijzonder patiënten met acromegalie. In 1982 slaagden twee, onafhankelijk van elkaar in hetzelfde instituut werkende, onderzoekers (Vale c.s. en Guillemin c.s.) er in de structuur van GHRH op te helderen. Drie peptiden welke in vitro de afgifte van groeihormoon (GH) door geïsoleerde ratte-hypofyse-cellen konden bevorderen, werden geïsoleerd uit pancreastumoren afkomstig van twee patiënten met acromegalie. Deze peptiden bevatten respectievelijk 44, 40 en 37 aminozuren ($GHRH_{44}$, $GHRH_{40}$, en $GHRH_{37}$). Aangetoond werd dat in de hypothalamus van de mens twee vormen van GHRH voorkomen, nl. $GHRH_{44}$ en $GHRH_{40}$ met een structuur identiek aan die van de ectopische geproduceerde peptiden. In een overzicht van de literatuur (tot en met juli 1987) worden in Hoofdstuk 1 de in vitro en in vivo experimenten met GHRH, bij proefdieren en bij de mens, beschreven.

Om na te gaan of er een geslachtsverschil bestaat in de groeihormoonafgifte werd het effect onderzocht van toediening van een i.v. bolus $GHRH_{44}$ (100 µg) aan jonge volwassen mannen en vrouwen (gemiddelde leeftijd 23 jaar) (Hoofdstuk 2.1). Zowel bij de mannen als bij de vrouwen werden maximale GH waarden binnen 45 minuten na de injectie bereikt. Bij beide groepen werd bovendien een geringe stijging van het prolactine-gehalte waargenomen, welke afwezig was na toediening van een zoutoplossing. Het antwoord van GH op toediening van GHRH was bij de mannen 45 minuten na de injectie significant hoger dan bij de vrouwen. Ook het maximale antwoord van GH op GHRH en de oppervlakte onder de GH-curve waren bij de mannen significant hoger dan bij de vrouwen. De fase van de menstruele cyclus bleek geen invloed te hebben op het antwoord van GH. Bij ratten werd een vergelijkbare invloed van het geslacht op de GH-afgifte na GHRH, zowel in vivo als in vitro, vastgesteld: androgenen blijken een stimulerend effect te hebben, terwijl oestrogenen het antwoord niet beïnvloeden. Ook bij de mens zou een dergelijk mechanisme een rol kunnen spelen.

In Hoofdstuk 2.2 werd de invloed onderzocht van toediening van een i.v. bolus van 100 µg $GHRH_{44}$ aan te lange jongens en meisjes (lengte > 90ste percentiel, gemiddelde leeftijd 14.5 jaar). De fase van de puberteit was voor beide groepen ongeveer vergelijkbaar. Het bleek dat op alle gekozen tijdstippen, tot 30 minuten na de injectie, het antwoord van GH op toediening van GHRH bij de meisjes groter was dan bij de jongens. Ook de maximaal bereikte GH-spiegels waren bij de (te lange) meisjes ongeveer twee maal hoger dan bij de jongens. Een duidelijke verklaring voor de gevonden verschillen in het GH-antwoord op toediening van GHRH tussen jonge volwassen mannen en vrouwen enerzijds, en tussen te lange meisjes en jongens an-

derzijds is moeilijk te geven. Vergelijkbare verschillen werden overigens ook gevonden bij de rat: in de puberteit is het GH-antwoord op GHRH bij vrouwelijke dieren sterker, terwijl bij volwassen dieren juist het omgekeerde het geval is.

Het feit dat het antwoord op GHRH in vitro wordt gestimuleerd door glucocorticoiden, terwijl daarentegen behandeling van kinderen met corticosteroiden tot groeiremming leidt, vormde de aanleiding het effect van GHRH op de GH-afgifte te onderzoeken bij patienten met hypercortisolisme door Cushing syndromen (Hoofdstuk 3). Zowel patienten met de ziekte van Cushing als patienten met een primair adrenaal hypercortisolisme lieten een duidelijk verminderd of geheel afwezig antwoord van GH op GHRH zien. Het is al lang bekend dat bij hypercortisolisme remming van de GH-secretie optreedt Het onderzoek in Hoofdstuk 3 leert dat de verminderde stimuleerbaarheid van de GH-secretie door GHRH hiertoe bijdraagt.

In Hoofdstuk 4 wordt het effect van GHRH-toediening op de afgifte van GH door de hypofyse bij patienten met acromegalie besproken. Allereerst werd nagegaan of er een relatie bestaat tussen de grootte van de sella, het basale GH-gehalte en het antwoord van GH na toediening van GHRH (Hoofdstuk 4 1). Een duidelijke correlatie bestond tussen de grootte van de sella enerzijds, en zowel het basale GH-gehalte als de door GHRH-geinduceerde GH-stijging anderzijds. Ook in deze studie bleek de basale GH-spiegel in sterke mate het antwoord op GHRH te bepalen, zoals eerder was gerapporteerd (zie Hoofdstuk 4.4). Werden de patienten ingedeeld op grond van hun geslacht, dan werden statistisch significante relaties alleen bij de mannen gevonden, echter niet bij de vrouwen. Deze merkwaardige bevinding wordt in de discussie van Hoofdstuk 4.1 van enig commentaar voorzien.

Chiodini et al. verdedigden de stelling, dat GH-producerende adenomen zijn opgebouwd uit cellen met eigenschappen die passen bij zuiver somatotrope dan wel lactotrope cellen. Daarom werd het effect van GHRH, TRH en bromocriptine (Br)-toediening op de afgifte van GH bij 31 patienten met acromegalie onderzocht (Hoofdstuk 4.2). Het antwoord van GH op een i.v. bolus injectie van GHRH (100 µg) was erg wisselend (ΔGH 1-995 ng/ml), maar correleerde sterk met het basale GH-gehalte. Een negatieve relatie werd gevonden tussen de maximale procentuele (%) daling van het GH-gehalte na Br-toediening en de maximale % GH-stijging na GHRH, met andere woorden, hoe minder GH stijgt onder invloed van GHRH des te sterker is de daling na Br. Tevens werd een omgekeerde relatie vastgesteld tussen de maximale % GH-stijging na GHRH en na TRH. De reeds langer bekende samenhang tussen de uitkomsten van de GH-stijging na TRH en de daling onder invloed van Br kon worden bevestigd. Deze gegevens steunen de hypothese van Chiodini over het bestaan van GH-producerende adenomen die vooral reageren op GHRH, maar minder op TRH en Br ("pure somatotroph adenomas") en van gemengde adenomen die vooral gevoelig zijn voor TRH en Br, maar minder voor GHRH ("lacto-

troph-like adenomas").

Vervolgens werd nagegaan of lijders aan acromegalie die op TRH reageren met een paradoxaal antwoord van GH ("TRH-responders") en dus wellicht een adenoom hebben met vooral lactotrope eigenschappen, verschillen van patienten die geen duidelijk antwoord op TRH vertonen ("pure somatotroph adenomas") in hun GH antwoord op LHRH, Br en somatostatine (SRIF) (Hoofdstuk 4.3). Statistisch significante correlaties tussen de antwoorden van GH op GHRH, TRH en Br werden alleen gevonden bij de TRH-responders, niet bij de "non-responders". De groep die niet op TRH reageerde met een paradoxale GH-stijging toonde echter wel significante correlaties tussen zowel de maximale % GH-stijging na GHRH en LHRH als tussen de % GH toename na LHRH en de % daling na SRIF. Geen statistisch significante relatie werd gevonden tussen de stijging van GH op GHRH en de daling op SRIF-infusie, noch in de groep van de TRH-responders noch bij de non-responders De % GH-daling na toediening van SRIF was voor beide groepen gelijk, terwijl de GH-daling na Br, zoals te verwachten, meer was uitgesproken in de TRH responder- dan in de non-responder-groep. Tevens werd een duidelijke samenhang gevonden tussen de antwoorden van GH en prolactine. Zo bleek de % afname van het GH-gehalte na Br groter te zijn naarmate het basale prolactine-gehalte hoger was terwijl de % toeneming van het prolactine-gehalte na TRH hoger was naarmate de % prolactine-stijging na GHRH meer was uitgesproken. De laatst genoemde correlatie was alleen aanwezig bij de TRH-responders.

Uit het bovenstaande blijkt dat patienten met acromegalie op grond van hun reactiepatroon op stimuli en remmers van de GH-afgifte kunnen worden onderverdeeld in patienten met GH producerende adenomen met meer somatotrope en patienten met adenomen die meer lactotrope eigenschappen hebben deze notie zou van betekenis kunnen zijn met betrekking tot de keuze van de behandeling bij deze groep patienten.

In Hoofdstuk 4.4 worden de effecten van GHRH op de afgifte van GH,al dan niet na gelijktijdige toediening van SRIF beschreven. Het maximale antwoord van GH werd bereikt binnen 20 minuten na de toediening van GHRH. Zoals boven reeds vermeld was het antwoord van GH zeer wisselend, maar bleek het des te sterker naarmate het basale GH-gehalte hoger was. Infusie van SRIF (300 µg/uur), 1 uur voor toediening van GHRH, had een sterke daling van GH-spiegels tot gevolg. Toediening van GHRH tijdens SRIF-infusie deed bij 3 van de 4 patienten, bij wie het GH-gehalte volledig was genormaliseerd, het antwoord van GH verdwijnen. Voor de gehele groep van patienten gold dat het antwoord van GH op GHRH wel was verminderd, maar dat nog steeds de mate van het antwoord werd bepaald door de hoogte van het GH-gehalte gevonden 1 uur na de start van de SRIF-toediening. Deze waarneming is een sterke aanwijzing dat primair gebrek aan SRIF of primaire overproduktie van GHRH zelden of nooit aan acromegalie van niet-ectopische

origine ten grondslag ligt.Dus zowel met als zonder SRIF wordt het antwoord van GH op GHRH bepaald door de uitgangsspiegel van GH. Men kan veronderstellen dat de toenemende reactiviteit van GH op GHRH bij hogere GH-spiegels het gevolg is van een toegenomen aantal GH producerende cellen bij acromegalie. In dit kader past ook de bevinding van een groter sellavolume bij patienten met hogere GH-spiegels (zie boven). Het aantal somatotrope cellen bepaalt dus wellicht het basale GH-gehalte. Onze conclusie is dan ook dat de afgifte van GH na toediening van GHRH en SRIF aan patienten met acromegalie in wezen normaal is, zij het op een hoger niveau.

De effecten van toediening van een langwerkend analoog van SRIF (minisomatostatine, SMS 201-995) op de GH kinetiek, bij patienten met acromegalie worden beschreven in Hoofdstuk 4.5. Bij 8 van 12 patienten normaliseerden na toediening van 50 µg SMS subcutaan de GH-spiegels binnen 4 uur. Conform de bevindingen met SRIF gaf toediening van SMS 1 uur voor GHRH injectie aanleiding tot een sterk verminderd GH-antwoord. Na SMS-behandeling gedurende 1 maand bleken de GH-antwoorden op GHRH en op TRH bij de meeste patienten sterk te zijn gedaald of verdwenen Bij de 4 patienten met een paradoxaal GH-antwoord op LHRH verdween dit abnormale antwoord echter niet. In dit kader is het interessant te vermelden dat in de literatuur na chronische behandeling van acromegalie met Br geen verminderd antwoord van GH op GHRH werd gevonden, maar dat het abnormale GH-antwoord na TRH ongeveer halveerde en het paradoxale GH-antwoord op LHRH volledig verdween. Het lag daarom ook voor de hand SMS en Br te combineren bij de behandeling van acromegalie. Deze waarnemingen kunnen als aanwijzing worden gezien voor het standpunt om bij de behandeling van acromegalie van de combinatie van Br en SMS meer te verwachten dan van de behandeling met elk van deze medicamenten afzonderlijk.

SUMMARY

This thesis describes the effects of iv bolus administration of growth hormone-releasing hormone (GHRH) in healthy young adult men and women, in tall statured pubertal boys and girls, in patients with Cushing's syndrome and more specifically in acromegalics. Until 1982, GHRH was the last postulated releasing hormone of which the primary structure was unknown. In that year two groups of researchers (Vale et al. and Guillemin et al.) unraveled its structure from an ectopic source of GHRH. Three peptides with GH releasing activity i.e. $GHRH_{44}$, $GHRH_{40}$ and $GHRH_{37}$ were isolated and identified in pancreatic tumors from two patients with acromegaly. It was shown that in the human hypothalamus two forms of GHRH exist: $GHRH_{44}$ and $GHRH_{40}$ with a structure identical to that of the tumor peptides.

To study the influence of sex in GH responsiveness to GHRH we investigated the effects of an acute iv bolus administration of 100 μg $GHRH_{44}$ to young adult men and women in Chapter 2.1. Maximum GH levels were reached within 45 minutes both in the men and the women. The GH response to GHRH 45 minutes after the injection, the maximum GH increments and the areas under the GH response curve were significantly higher in the men than in the women. In the women no differences in maximum GH responses after GHRH were found between the follicular and luteal phases of the menstrual cycle. Serum prolactin levels slightly but significantly increased in both the men and the women within 5 minutes after injection of GHRH (not after placebo). In vivo and in vitro data of GH responsiveness to GHRH in rodents reveal a similar sex difference and an enhancing effect of androgens, but not of estrogens. Therefore, also in humans testosterone may play a role in the genesis of the sex difference in GH responsiveness to GHRH.

Subsequently we investigated (Chapter 2.2) the GH response to a bolus injection of 100 μg $GHRH_{44}$ in midpubertal tall girls and tall boys (height > P 90). It appeared that at all time intervals up to 30 minutes after the bolus injection, the GH responses to GHRH were significantly higher in the girls than in the boys and the peak GH increments to GHRH were about twice as high in the former than in the latter. No ready explanation can be given for the observed sex related differences in GH responsiveness to GHRH between tall statured pubertal boys and girls, which contrast with that found in young adult men and women. Similar findings, however, have been reported in young rats, i.e. higher GH responses to GHRH in the females than in the males. In adult rats the higher GH responses are found in the males.

The stunting of growth in children with glucocorticoid excess prompted us to investigate GH responsiveness to GHRH in patients with endogenous hypercortisolism (Chapter 3). An absent or severely blunted GH response to

GHRH was found in all patients with Cushing's disease and those with hypercortisolism due to an adrenocortical adenoma.

In Chapter 4 studies on GHRH testing in patients with acromegaly are reported. The first question we wanted to address is, whether there are relationships between the basal GH, sellar volume (SV) and the GH response to GHRH in acromegalics (Chapter 4.1). In the whole group of acromegalics the basal GH levels and SV were directly correlated i.e. the larger SV, the higher basal GH level. SV was also correlated with the absolute GH increments in response to GHRH. The latter was tightly related to the basal GH level. However, subdividing the patients according to sex, only in the men a close relation was found between SV and both basal and GHRH-stimulated GH, not in the women. One might speculate that the well-known protective effect of estrogens on the action of GH in peripheral tissues is responsible for the observed differences, thereby probably delaying the expression of GH excess in women. The precise mechanisms underlying this sex difference await further elucidation, especially the modulatory effects of gonadal steroids.

To verify the hypothesis of the existence of GH producing adenomas in acromegaly with more somatotroph or more lactotroph properties we compared the effect of GHRH, TRH and bromocriptine (Br) on GH levels in 31 patients with acromegaly (Chapter 4.2). The GH response to $GHRH_{44}$ was highly variable (Δ GH 1-995 ng/ml), but strongly correlated with basal GH levels. We found an inverse relation between the percentage (%) maximum GH decrease after Br and the % peak GH response to GHRH i.e. the lower the GH increase after GHRH, the more pronounced the GH decrease after Br. Furthermore, a reciprocal relation was found between the % peak GH response to GHRH and to TRH. The already known relation between the % GH decrease after Br and the % peak GH responses to TRH could be confirmed. The data therefore are consistent with Chiodini's hypothesis of the existence of GH secreting adenomas which are more sensitive to GHRH and less to TRH and Br (pure somatotroph adenomas) and of mixed (lactotroph-like) adenomas responsive to TRH and Br, but less to GHRH.

The next study (Chapter 4.3) was designed to investigate whether patients responding with a paradoxical GH increase after TRH with allegedly lactotroph-like adenomas differ from those patients non-responding to TRH with more somatotroph-like tumors, in their GH answers to GHRH, LHRH, Br and somatostatin (SRIF). We could demonstrate that statistically significant relations between the GH responses to GHRH, TRH and Br are only present in TRH-responding acro-megalics, not however in TRH non-responders. In contrast, in these latter patients, not in the former, close relations were found between the % peak GH responses to LHRH and to GHRH and the % GH decrements in response to SRIF infusion. No statistically significant relation was found between the % GH responses to GHRH and to SRIF,

neither in the TRH non-responders, nor in the TRH responders. The % GH response to Br was as expected significantly higher in the TRH responders than in the TRH-non-responders, although also in these patients the GH decrease was substantial. Furthermore, close correlations were found between the prolactin and GH data. The % GH decrease in response to Br was the more pronounced, the higher the basal serum prolactin levels, whereas the % peak prolactin response to TRH was the higher, the higher the % peak prolactin response to GHRH. Only in the TRH responder group basal serum prolactin levels and the GH decrements to Br were tightly correlated, as were the prolactin responses to TRH and GHRH. The data further favour the concept of the existence of subgroups of acromegalics with a more "lactotroph-" or "somatotroph"-like adenoma with corresponding GH responses to releasing and inhibiting agents. Firm conclusions with respect to the choice of therapy in the individual patient can, however, not be drawn.

In Chapter 4.4 we describe the effects of iv injection of 100 μg $GHRH_{44}$ and/or concomitant SRIF infusion to 11 patients with acromegaly. The maximum GH response to GHRH was achieved 20 minutes after the injection and was highly variable but closely correlated with basal GH levels. SRIF started one hour before GHRH injection (300 μg/hr) expectedly lowered basal GH levels. GHRH injection (100 μg) while con-tinuing SRIF infusion for another hour led to completely blunted GH responses in 3 out of 4 patients whose GH levels normalized during SRIF infusion. Maximum GH responses to GHRH and concomitant SRIF infusion were highly variable but again positively correlated with the GH levels before GHRH testing i.e. 1 hour after SRIF infusion. It is intriguing to note that GH responsiveness to GHRH is determined by the basal GH level both with and without concomitant SRIF infusion. One might speculate that the increased responsiveness to GHRH at higher GH levels is the consequence of an increased number of GH-producing cells in acromegaly. The finding of a larger sella in acromegalic patients with the higher GH responses to GHRH is in line with such thesis. By a similar way of reasoning the number of somatotrophs may indicate the basal GH level. Therefore, we can conclude that responsiveness to GHRH and SRIF is essentially normal in acromegaly, but at a higher setting of basal GH levels.

The effects of minisomatostatin (SMS), a long acting analogue of SRIF, on GH kinetic in acromegaly are described in Chapter 4.5. SMS at a dose of 50 μg subcutaneously normalized GH levels 4 hours after the injection in 8 out of 12 patients. As was earlier found with SRIF, GH responsiveness to GHRH was strongly blunted when SMS was administered 1 hour before GHRH injection. The GH response to GHRH and the paradoxical GH responses to TRH disappeared in the majority of patients after SMS treatment for 1 month. In contrast paradoxical GH responsiveness to LHRH persisted in 4 out of 4 patients. It is interesting to note that other authors reported that

chronic treatment with Br had no effect upon the GH answer to GHRH, reduced the paradoxical GH responses to TRH with approximately 50% and completely blunted the anomalous GH responses to LHRH. Therefore SMS and Br may have complementary effects in correcting the anomalous GH kinetics in patients with acromegaly. Combination of the two drugs therefore may be justified in treating acromegalics and in fact has been reported to be more effective than each of the drugs alone in selected patients.

DANKWOORD

Mijn dank gaat allereerst uit naar de patiënten en vrijwilligers die bereid waren de verschillende testen te ondergaan.

De testen op de afdeling werden op deskundige wijze uitgevoerd onder leiding van Mevr.Zr.C. Willemsen en Mevr.Zr. T. Terburg.

Verder wil ik de medewerkers van de afdeling Experimentele en Chemische Endocrinologie (Hoofd: Prof.Dr. Th. J. Benraad) danken voor het bepalen van de vele groeihormoon- en prolactine-monsters.

Mevr.Drs. W. Buijs ben ik zeer erkentelijk voor de statistische en de computerbewerking van de gegevens.

Dank ook aan de medewerkers van de Medische Bibliotheek (Hoofd: Mevr. Drs. S. Bakker) voor hun hulp bij het verzamelen van de literatuurgegevens.

De medewerkers van de afdeling Medische Oncologie (Hoofd: Prof.Dr. D. J. Th. Wagener) dank ik voor de uren die zij voor mij waarnamen, als er weer eens een artikel besproken moest worden.

De vele versies van dit proefschrift werden met nimmer aflatende toewijding uitgewerkt door Mevr.W. Straten en Mevr.I. Swinkels-Scholten.

Last but not least wil ik mijn vrouw danken voor het uitwerken van de vele literatuurreferenties en het aanhoren van de beslommeringen die de geboorte van een proefschrift met zich meebrengen.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren te Herten op 14 augustus 1955. Het diploma HBS-B werd gehaald aan het Bisschoppelijk College te Roermond in 1972. In datzelfde jaar werd begonnen met de medische studie aan de Katholieke Universiteit te Nijmegen, die in 1977 werd afgesloten met het doctoraal examen en in december 1979 met het artsexamen. Op 1 februari 1980 werd begonnen met de opleiding tot internist in het St. Jozefziekenhuis te Eindhoven (Hoofd: Dr. P. Deckers). Op 1 november 1981 werd deze opleiding vervolgd op de afdeling Interne Geneeskunde van het Sint-Radboudziekenhuis te Nijmegen (Hoofd: Prof.Dr. A. van 't Laar). Op 1 februari 1985 werd hij ingeschreven in het Specialistenregister. In 1984 werd gestart met het onderzoek naar de klinische betekenis van groeihormoon-releasing hormoon op de afdeling Endocriene Ziekten (Hoofd: Prof. Dr. P. W. C. Kloppenborg). Vanaf 1 april 1985 tot 1 november 1987 was de auteur, met een onderbreking van 10 maanden op de afdeling Endocriene Ziekten, werkzaam op de afdeling Medische Oncologie van het Sint-Radboudziekenhuis (Hoofd: Prof.Dr. D. J. Th. Wagener). De komende 2 jaar wordt hij door het Koningin Wilhelmina Fonds via een klinisch fellowship in staat gesteld zich verder te bekwamen in de oncologie.

STELLINGEN

1. Ook bij de mens lijkt er een geslachtsverschil te bestaan in het antwoord van groeihormoon op de toediening van groeihormoon-releasing-hormoon.
   Dit proefschrift

2. Bij hypercortisolisme is het antwoord van groeihormoon op groeihormoon-releasing-hormoon afwezig dan wel sterk verminderd.
   Dit proefschrift

3. Het antwoord van groeihormoon op groeihormoon-releasing-hormoon bij patiënten met acromegalie is hoger naarmate het antwoord op thyrotropin-releasing-hormoon en bromocriptine lager is.
   Dit proefschrift

4. Patienten met acromegalie kunnen op grond van hun reactiepatroon op stimuli en remmers van de groeihormoon-afgifte worden onderverdeeld in patienten met adenomen met meer somatotrope en patienten met adenomen die meer lactotrope eigenschappen hebben. Deze bevinding heeft evenwel voor de individuele patient nauwelijks betekenis met betrekking tot de keuze van de behandeling.
   Dit proefschrift

5. Aanwezigheid van de epidermal growth factor receptor in mammaweefsel bepaalt in belangrijke mate het optreden van vroege recidieven en/of de overleving bij patienten met een primair mammacarcinoom. Sainsbury et al. The Lancet 1987 i: 1398-1402.

6. Het verband tussen roken en het optreden van endocriene ophthalmopathie dient nader te worden onderzocht (Hägg & Asplund, Brit Med J, 1987, 296: 634-635.)

7. Toediening van hoge doses glucocorticoïden aan patienten met septische shock lijkt achterhaald. (Bone et al. New Engl J Med 1987, 317: 653-659). Deze stelling, onlangs nog eens door goed onderzoek onderbouwd, had 20 jaar geleden eveneens kunnen zijn geponeerd.

8. Evenals bij het varken is ook bij de mens androstadienol de belangrijkste voorloper van sex-feromoon (Weusten et al. J Clin Endocrinol Metab, oct. 1987).

9. De uitspraak: "Geen nieuws is goed nieuws" geldt niet voor een sollicitatie.

10. Als de overheid als een ontgrondingsbedrijf en de ontgrondingsbedrijven als een overheid geleid zouden worden, dan zouden alle ontgrondingsbedrijven failliet zijn en was Nederland één grote waterplas.

11 Ook al zou de Rijn in het jaar 2000 geheel schoon zijn, dan nog is het onwaarschijnlijk dat de zalm hierin terugkeert.

12 De hoofdhuid leent zich uitermate voor onderzoek naar het effect van locaal te appliceren experimentele geneesmiddelen: bij behandeling van alopecia areata verdient het dan ook aanbeveling de ene schedelhelft wel en de andere niet te behandelen (Happle).

13. Het is aannemelijk dat tussen nu en het jaar 2000 het aantal kankerpatienten zal toenemen van 200.000 tot 300.000 Wil men de problematiek die hier mee gepaard gaat opvangen, dan dient nu actie te worden ondernomen (S.T.G. rapport Kanker in Nederland, 1987).

14 Het wordt de hoogste tijd een "stellingenbank" voor promovendi op te richten.

Nijmegen 4 december 1987